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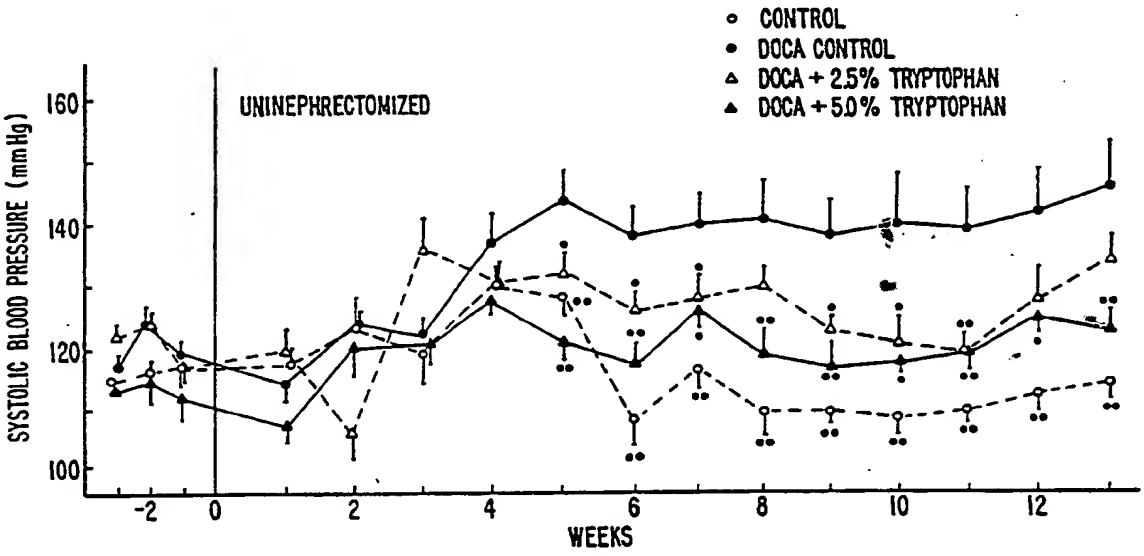
(54) Title: TREATMENT OF HYPERTENSION BY CHRONIC ADMINISTRATION OF L-TRYPTOPHAN OR L-5-HYDROXYTRYPTOPHAN



(57) Abstract

A composition and method for treating hypertension, cardiac or renal hypertrophy, polydipsia, polyuria, stroke or atherosclerosis based on chronic administration of L-tryptophan or L-5-hydroxytryptophan.

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Treatment of hypertension by chronic administration of L-tryptophan or L-5-hydroxytryptophan

BACKGROUND OF THE INVENTION

Related Applications

This is a continuation-in-part application of Serial No. 102,723, filed September 30, 1987.

Field of the Invention

The present invention relates to a method of treating hypertension. The work leading to the filing of the present application was supported by Grant HL 29459 from the National Institutes of Health.

Prior Art

The treatment of high blood pressure has not heretofore been satisfactory. Although some drugs have been found effective to lower blood pressure, the side effects are such that the major question is whether the desirability of lowering blood pressure is outweighed by the detrimental effects of the drug.

The majority of human patients with an elevated blood pressure fall into the categories of borderline or mild hypertension. A significant concern at the present time relates to the necessity of instituting drug therapy in such patients. It is therefore desirable to explore alternative possibilities to control blood pressure, including an understanding of the fundamental processes of nutrition in hypertension. From such understanding, nutritional interventions may then be instituted logically prior to initiation of any drug therapies.

There have been studies which have demonstrated that the acute administration of tryptophan to certain spontaneously hypertensive animals (rats) could lower their blood pressures. [Ito et al, In: Central Nervous System Mechanisms

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Responsible for Blood Pressure Elevations Induced by P-chlorophenylalanime. J. Pharmac. Exp. Ther. Vol. 181, pp. 65-74 (1972); Jarrott et al, In: Serotonin Levels in Vascular Tissue and the Effects of a Serotonin Synthesis Inhibitor on Blood Pressure of Hypertensive Rats, Clin. Exp. Pharmacol. Physiol. Suppl. 2, pp. 201-205 (1975); Fuller et al, In: Antihypertensive Effects of Fluoxitine and L-5-hydroxytryptophan in Rats, Life Sci., Vol. 25, pp. 1237-1242 (1979); Sved et al, In: Studies on the Antihypertensive Action of L-tryptophan, J. Pharmac. Exp. Ther., Vol. 221, pp. 329-333 (1982); Wolf et al, In: Effects of L-tryptophan on Blood Pressure in Normotensive and Hypertensive Rats, J. Pharmac. Exp. Ther., Vol. 230, pp. 324-329 (1984); and U.S. Patent No. 4,224,343].

The effectiveness of the acute administration of a drug to treat a condition cannot be reliably and predictably extrapolated into a conclusion that the chronic administration of the same drug will produce similar results. This is illustrated by the fact that, although acute administration of tyrosine can reduce blood pressure in rats, chronic administration does not [See Lockley, et al, In: Effect of 1-p-tyrosine on the Development of Renal Hypertension in Rats, Pharmacology Vol. 31, pp. 132-149 (1985); Henley et al, In: Physiologic Responses to Chronic Dietary Tyrosine Supplementation in DOCA-salt-treated Rats, Pharmacology Vol. 33, pp. 334-347 (1986); Sole et al, In: Chronic Dietary Tyrosine Supplements Do Not Affect Mild Essential Hypertension, Hypertension Vol. 7, pp. 593-596 (1985)].

There is also disagreement with regard to the mechanism(s) by which treatment with tryptophan

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reduces blood pressure both acutely and chronically. [Ito et al, In: Central Nervous System Mechanisms Responsible for Blood Pressure Elevations Induced by P-chlorophenylalanine, J. Pharmac. Exp. Ther., Vol. 181, pp. 65-74 (1972); Wing et al, In: Effects of P-chlorophenylalanine on Blood Pressure and Heart Rate in Normal Rabbits and Rabbits with Neurogenic Hypertension, Clin. Exp. Pharmacol., Vol. 1, pp. 219-229 (1974); Chalmers et al, In: Central Serotonin and Cardiovascular Control, Clin. Exp. Pharmacol. Physiol. Suppl. 2, pp. 195-200 (1975); Finch, L., In: The Cardiovascular Effects of Intraventricular 5, 6-dihydroxytryptamine in Conscious Hypertensive Rats, Clin. Exp. Pharmacol. Physiol., Vol. 2, pp. 503-508 (1975); Jarrott et al, In: Serotonin Levels in Vascular Tissue and the Effects of a Serotonin Synthesis Inhibitor on Blood Pressure of Hypertensive Rats, Clin. Exp. Pharmacol. Physiol. Suppl. 2, pp. 201-205 (1975); Buckingham et al, In: Effect of Intracerebroventricular 5, 6-dihydroxytryptamine on Blood Pressure of Spontaneously Hypertensive Rats, Europ. J. Pharmacol., Vol. 36, pp. 431-437 (1976); Fuller et al, In: Antihypertensive Effects of Fluoxitine and L-5-hydroxytryptophan in Rats, Life Sci., Vol. 25, pp. 1237-1242 (1979); Sved et al, In: Studies on the Antihypertensive Action of L-tryptophan, J. Pharmac. Exp. Ther., Vol. 221, pp. 329-333 (1982); Wolf et al, In: Effects of L-tryptophan on Blood Pressure in Normotensive and Hypertensive Rats, J. Pharmac. Exp. Ther., Vol. 230, pp. 324-329 (1984)]. The maximal reduction in the blood pressure (30 mm Hg) of rats administered 50 mg tryptophan/kg, i.p., occurred within two hours after acute treatment and returned to pretreatment level within four hours.

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U.S. Patents 3,150,044 and 3,298,916 relate to the treatment of hypertension and report that the administration of L-tryptophan and reserpine act synergistically to produce a lowering of blood pressure. These patents also contain a statement that "L-tryptophan has some significant effect (on hypertension) by itself and can be supplied to the human system in amounts of as much as 2 grams per day --- It (being) preferred to limit the daily dosage to the human body of L-tryptophan to an amount within the range of 1/16 - 1 gram per day." There are, however, no data or evidence presented in the patent, other than the inventor's unsupported allegations, to buttress the claims of the efficiacy of administration of L-tryptophan to prevent or lower blood pressure. Rather, the entire thrust of the patents referred to above is to emphasize the effectiveness of a combination of L-tryptophan and reserpine [or other specific agents] to treat hypertension.

It is an object of the present invention to provide a method and composition for the treatment of hypertension involving the chronic administration to a patient in need thereof of a compound having substantially little or no toxicity or adverse side effects.

SUMMARY OF THE INVENTION

The above and other objects are achieved by the present invention which provides a method for the treatment of hypertension in a human patient or non-human animal subject requiring such treatment consisting essentially of chronically administering a daily dosage of from about 4 to about 10 grams/day (60-150 mg/kg of body wt.) of L-tryptophan or

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L-5-hydroxytryptophan for a time sufficient to significantly lower the blood pressure.

The invention further provides a method for the prevention of certain conditions accompanying hypertension, such as cardiac hypertrophy, renal hypertrophy, polydipsia, polyuria, stroke and atherosclerosis in the human requiring such treatment consisting essentially of chronically administering a daily dosage of from about 4 to about 10 grams/day of tryptophan for a time sufficient to ameliorate the condition.

The invention also provides a pharmaceutical composition in unit dosage form adapted for the chronic administration to a human in need of treatment of hypertension and prevention of the conditions listed above consisting essentially of an amount of L-tryptophan or L-5-hydroxytryptophan such that the administration of the composition to the patient will comprise a total daily dosage of from about 4 to about 10 grams/day of L-tryptophan or L-5-hydroxytryptophan and a pharmaceutically acceptable carrier therefor.

Finally, the invention provides a dietary supplement composition including pharmacologically acceptable salts in unit dosage form adapted for the chronic administration to a human in need of treatment of hypertension and the other conditions listed above consisting essentially of an amount of L-tryptophan such that the administration of the composition will comprise a total daily dosage of from about 4 to about 10 grams/day of L-tryptophan or L-5-hydroxytryptophan and an acceptable dietary carrier therefor.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-14 are graphic representations of the results of several of the examples set forth herein below which illustrate the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated on the surprising discovery that the chronic administration of much larger dosages of L-tryptophan or L-5-hydroxytryptophan than heretofore reported are required for successfully treating hypertension and other cardio-related diseases or conditions in human and non-human animals.

Moreover, it has been unexpectedly discovered that the L-tryptophan or L-5-hydroxy-tryptophan may be administered in either pharmaceutical composition or dietary supplement form to achieve the above-described benefits.

It is preferred to administer a daily dosage of from about 4 to about 6 grams/day of tryptophan or L-5-hydroxytryptophan for the treatment of hypertension in humans and from about 4 to about 6 grams/day for the prevention of other cardiac, renal and cerebral vascular conditions noted above.

Although the invention is primarily intended for the treatment of human beings, it will be understood by those skilled in the art that it may also be used to treat non-human animals, e.g., dogs, cats and other domesticated animals.

The daily dosage may be divided among multiple administrations, either in pharmaceutical composition form or as a dietary supplement.

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L-tryptophan may be compounded in capsule, tablet or other orally administrable form utilizing such pharmaceutically acceptable carriers as sugars, e.g., lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, as well as binders such as starch, pastes (using maize starch, wheat starch, rice or potato starch); or it may be combined with carriers suitable for parenteral administration [i.v., intra-muscular, etc.] such as push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compound in the form of granules, e.g., mixed with filler such as lactose, binders such as starches and/or lubricants such as talc or magnesium stearate and, optimally, stabilizers.

L-tryptophan may be compounded with the above described carriers according to conventional methods.

Alternatively, L-tryptophan or L-5-hydroxy-tryptophan may be administered as a dietary supplement in certain foods and drinks which are low in protein composition.

DETAILED DESCRIPTION OF THE DRAWINGS

In the drawings:

in FIG. 1A, chronic dietary administration of L-tryptophan (25 and 50 g/kg of food) prevented the development of DOCA-salt-induced hypertension in rats. The vertical line designates the time unilateral nephrectomy took place. One standard error is set off at each mean. Symbols representing the four groups (8 rats/group) are designated in the figure.

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* = P<0.05; ** = P<0.01 compared to the DOCA-treated group. In FIG. 1B, the mean body weights of the four groups shown in A are depicted. Treatment with either DOCA or DOCA with tryptophan had no significant effect on body weight.

In FIG. 2, mean daily intake of isotonic saline (left panel), output of urine (middle panel), and intake of food (right panel) are shown. The four groups (8 rats/group) are designated in the figure. Treatment with DOCA increased intake of saline and output of urine significantly above those of controls while graded doses of tryptophan given to DOCA-treated rats reduced intake of saline and output of urine in a graded fashion toward the level of controls. Intake of food was unaffected by any treatment. One standard error is set off at each mean. ** = P<0.01 compared to the control group.

In FIG. 3, mean daily rate of excretion of dopamine is shown in relation to mean daily rate of excretion of sodium by the three DOCA-treated groups. Each dot represents data from an individual rat. The regression equation and correlation coefficient are given in the figure.

In FIG. 4, the relationship between intake of isotonic saline and graded doses of AII are shown. Symbols representing the four groups (8 rats/group) are shown in the figure. Chronic treatment with DOCA increased significantly the drinking response to administered AII. Concurrent treatment with the higher dose of tryptophan reduced the exaggerated drinking response of the DOCA-treated group to the level of the control group. One standard error is set off at each mean.

In FIG. 5, the response of heart rate to administration of isoproterenol (5 µg/kg, s.c.) is

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shown for the four groups (8 rats/group). Symbols designating each group are given in the figure. One standard error is set off at each mean.

In FIG. 6, the effect of chronic dietary administration of L-tryptophan (25 and 50 g/kg food) on the development of DOCA-salt-induced hypertension in rats from Example 2 is shown in A and mean body weights of each group are shown in B. One standard error is set off at each mean.* = $P<0.05$; ** = $P<0.01$ compared to the DOCA-treated group.

FIG. 7 shows the effect of acute administration of angiotensin II (100 µg/kg, s.c.) on tail skin (A) and colonic (B) temperatures of the four groups of rats designated in the figure. An exaggerated increase in tail skin temperature was observed in the DOCA-treated rats. Concurrent treatment with tryptophan reduced the response to the level of controls. Colonic temperatures of all rats were reduced following treatment with angiotensin II. One standard error is set off at each mean.

FIG. 8 shows the specific binding of angiotensin II (AII) to membranes from the diencephalic portion of the brains of rats from the four groups as shown at 0.25 nM AII (upper panel) and 1.00 nM AII (lower panel). The groups are designated in the figure. One standard error is set off at each mean. ** = $P<0.01$ compared to the DOCA-treated group.

FIG. 9 shows the effect of chronic dietary administration of 0.5 and 1.0% L-tryptophan on the elevation of systolic blood pressure (a) and body weight (b) of rats whose kidneys were bilaterally encapsulated with latex envelopes. Renal encapsulation and diets began at time 0. O = Encapsulation; ▲ = encapsulation + 0.5% tryptophan;

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Δ = encapsulation + 1% tryptophan; bars represent 1SE. *P<0.05; **P<0.01 compared to the encapsulated control group.

FIG. 10 shows the effect of chronic dietary administration of 2.5 and 5.0% L-tryptophan on the elevation of systolic blood pressure (a) and body weight (b) of rats whose kidneys were bilaterally encapsulated with latex envelopes. Renal encapsulation and diets begin after time 0. ● = Control; O = encapsulation; ▲ = encapsulation + 2.5 % tryptophan; Δ = encapsulation + 5% tryptophan; bars represent 1 SE. *P<0.05; **P<0.01 compared to the encapsulated control group.

FIG. 11 depicts the effect of the chronic treatment with L-5-hydroxytryptophan (L-5-HTP, 6.3 and 12.6 mg/day by osmotic minipump) on the development of DOCA-salt-induced hypertension (a) and body weight (b). Control (O); DOCA (●); DOCA + 6.3 mg L-5-HTP/day (Δ), and DOCA + 12.6 mg L-5-HTP(day) (▲). One standard error is set off at each mean. *P<0.05; **P<0.01 compared with the DOCA-treated group.

FIG. 12 depicts the specific binding of angiotension II(AII) to membranes from the diencephalic portion of the brains of rats from four groups are shown at 0.25 nM AII (upper panel) and 1.0 nM AII (lower panel). One standard error is set off at each man. **P<0.01 compared with the DOCA-treated group.

FIG. 13 depicts the effect of chronic treatment with L-5-HTP by osmotic minipump (4.2 and 8.4 mg/day) on the development of DOCA-salt-induced hypertension (a) and body weight (b). DOCA (●); DOCA-salt + 4.2 mg L-5-HTP/day (Δ), and DOCA-salt + 8.4 mg L-5-HTP/day (▲). One standard error is set

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powdered Purina Laboratory Chow (#5001) ad libitum. Infant nursing bottles with cast aluminum spouts were used as fluid containers [Lazarow, A., In: Methods for Quantitative Measurement of Water Intake, Methods Med. Res, Vol. 6, pp. 225-229 (1954)]. Food containers were spill-resistant and are described in detail by Fregly [Fregly, M.J., In: A Simple and Accurate Feeding Device for Rats, J. Appl. Physiol., Vol. 15, p. 539 (1960)]. The vivarium was maintained at 26 ± 1°C. and illuminated from 7 a.m. to 7 p.m. Unless otherwise designated, the rats were maintained three per cage.

Systolic blood pressures were measured from the tail using the technique described by Fregly [Fregly, M.J., In: Factors Affecting Indirect Determination of Systolic Blood Pressure of Rats, J. Lab. Clin. Med., Vol 62, pp. 223-230 (1963)] and a polygraph. A two to three week control period preceded initiation of the experiments. During this time, blood pressure and body weight of each rat were measured weekly.

At the end of the control period, the rats were divided randomly into four equal groups. Unilateral (left) nephrectomy was then carried out in all animals while they were anesthetized with pentobarbital (40 mg/kg, i.p.). At this time, two preweighed, 25 mm long Silastic tubes (#602-265), filled with deoxycorticosterone acetate were implanted s.c. between the shoulder blades of three of the four groups [Fregly et al, In: Effect of Chronic Administration of Deoxycorticosterone Acetate on Salt Appetite of Captopril-treated Rats, Endocrinology (Baltimore), Vol. 116. pp. 1391-1398, (1985)]. The remaining was implanted s.c. with an empty Silastic tube. At the end of the experiment, the tubes were removed, dried for 72 hours in a desiccator, and weighed on an analytical balance. Based on the change in weight of the tubes and the mean weight of the

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off at each mean, *P<0.05; **P<0.01 compared with the DOCA-treated control group.

FIG. 14 depicts the effect of chronic treatment with L-5-HTP (4.2 and 8.4 mg/day) on the dipsogenic responsiveness of DOCA-treated rats to angiotension II (50 µg/kg body weight s.c., left panel; 100 µg/kg body weight, s.c., right panel). One standard error is set off at each mean. Control (□); L-5-HTP (4.2 mg/day ▨) and L-5-HTP (8.4 mg/day ▨), *P<0.05 compared with DOCA-treated control group.

FIG. 15 depicts blood pressure over time in a control group of 25 human patients to whom a placebo was administered.

FIGS. 16 and 17 depict blood pressure as a function of time and dosage, respectively, in a human patient to whom tryptophan was administered.

FIGS. 18 and 19 depict blood pressure as a function of time and dosage, respectively, in another human patient to whom tryptophan was administered.

FIGS. 20 and 21 depict blood pressure as a function of time and dosage, respectively, in a third human patient to whom tryptophan was administered.

FIG. 22 depicts changes in blood pressure as a function of dosage during the first week of administration of tryptophan to human patients.

The invention is illustrated by the following non-limiting examples:

EXAMPLE 1

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN IN THE DEVELOPMENT OF DOCA-INDUCED HYPERTENSION

Thirty-two female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing initially from 180 to 225 g were used in each of the examples herein. The rats were provided with tap water and finely

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animals during the time the tubes were implanted, an average of 801 ± 46 (S.E.) µg DOCA/kg/day (249 ± 31 µg/day) were released from the tubes.

Immediately after nephrectomy and implantation of Silastic tubes, two of the three DOCA-treated groups received L-tryptophan mixed into the finely powdered Purina Laboratory Chow at concentrations of 25 and 50 g/kg of food, respectively. The remaining DOCA-treated group and the control group received the same food without tryptophan. All rats were allowed only 0.15 M. NaCl solution to drink.

Following unilateral nephrectomy, implantation of tubes containing DOCA, and initiation of the tryptophan-supplemented diet, blood pressures and body weights were measured weekly for 5 weeks. During the fifth week, the rats were kept individually in stainless steel metabolic cages. They were provided their usual diet and 0.15 M. NaCl solution to drink. Urine was collected in Erhlenmeyer flasks containing 1.0 ml 6 N HCl daily for three days. Urinary concentrations of norepinephrine, epinephrine, and dopamine were measured by high performance liquid chromatography (HPLC) with electrochemical detection as described by [Carlberg et al, In: Catecholamine Excretion and Beta-adrenergic Responsiveness in Estrogen-treated Rats, Pharmacology, Vol. 32, pp. 147-156 (1986); Henley et al, In: Physiologic Responses to Chronic Dietary Tyrosine Supplementation in DOCA-salt-treated Rats, Pharmacology, Vol. 33, pp. 334-347 (1986).]. In brief, urinary catecholamines were isolated on cation resin exchange columns (Biorex 70 cation resin), eluted with 2 M ammonium sulfate, adsorbed on alumina, and eluted from alumina with 0.1 M perchloric acid. Dihydroxybenzylamine hydrobromide was used as an internal standard. The mobile phase used was a 0.1 M. (pH 3.0) monochloroacetate buffer, containing 2 mM sodium EDTA, 600 mg of sodium octyle

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sulfate and 10% acetonitrile (v/v). The stable phase consisted of a C_{18}-uBondapack column. Flow rate was set at 1.2 ml/min and the electrochemical detector at 0.65 v. All samples collected in one day were extracted and analyzed together and the data normalized to a mg of creatinine excretion. Urinary creatinine concentration was measured by the method of Chasson et al [Chasson et al, In: Determination of Creatinine by Means of an Automatic Chemical Analysis, Am. J. Clin. Path., Vol. 35, pp. 83-88 (1961)]. Urinary sodium and potassium concentrations were measured by flame photometry using lithium as the internal standard.

During the sixth week of the study, all rats, maintained in individual metabolic cages, were dehydrated with food available for 24 hours. Body weight was measured prior to and at the end of dehydration. Urine was collected under light mineral oil. Osmolality (by vapor pressure osometry) and sodium and potassium concentrations (by flame photometry) of the urine excreted by each rat were also measured. At the end of the 24 hour period of dehydration, each rat was given a preweighed bottle of saline (26°C.), and fluid intakes and urine outputs measured hourly for two hours.

At the beginning of the seventh week of the experiment, the dipsogenic response to acute administration of angiotensin II (AII) was tested in all rats. At 9 AM on the day of the test, food and fluid were removed from all cages and each rat was weighed, administered AII (25 µg/kg, s.c.), and placed alone in a cage. A preweighed bottle of isotonic saline (26°C.) was placed on each cage and fluid intake measured at 0.5, 1.0, and 2.0 hours thereafter.

At the end of the seventh week of the experiment, a second study was carried out identically to that described above except that AII was

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administered at a dose of 50 µg/kg., s.c. At the beginning and end of the eighth week, two additional studies were carried out identically to that described above except that 100 and 200 µg AII/kg., s.c., respectively, were administered.

During the ninth week of treatment, the dipsogenic response to administration of the beta-adrenoceptor agonist, d,l-isoproterenol HCl (5 µg/kg, s.c.) was tested in all rats as discussed above for AII during the seventh week of the experiment.

During the eleventh week of the experiment, the responsiveness of heart rate to administration of d,l-isoproterenol HCl (5 µg/kg, s.c.) was assessed. Each rat was lightly anesthetized with ether and electrode paste applied to a hairless area of the ventral chest at the level of the heart and to one of the rear feet. Recording leads were attached with adhesive tape and the rat placed into a tunnel-type cage consisting of a wire mesh tunnel with wooden floor [Adolph et al, In: Multiple Factors in Thirst, Am. J. Physiol., Vol. 178, pp. 538-562 (1954)]. Upon recovery from anesthesia, heart rate of each rat was measured for one minute at five minute intervals during a one-half hour control period. If measurements of heart rate during the last 10 minutes agreed within 5%, the rat was injected with isoproterenol through a slot in the cage. Heart rate was then measured for one minute at each five minute interval for the first half hour and for one minute at each 10 minute interval thereafter until the study was terminated.

During the twelfth week of the experiment, the rats were placed individually into metabolic cages and given a choice between water and 0.15 M NaCl solution to drink. Intakes of food, water, and NaCl solution were measured daily for two days.

During the thirteenth week of the experiment, each rat was restrained in a plexiglass

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cylinder just large enough to hold it comfortably and its colonic temperature was measured using a probe inserted 5 cm into the colon for 30 seconds. Two days later, the rats were killed by decapitation and blood from the trunk collected in a beaker containing EDTA for measurement of plasma renin activity by radioimmunoassay. At death, heart, right kidney, adrenals, and thyroid gland were removed, cleaned of extraneous tissue and weighed on a torsion balance.

Statistical analysis of the data was carried out by means of an analysis of variance and a Newman-Keuls post hoc test to determine the difference between any two individual means [Snedecor et al, In: Statistical Methods, 5th Edition, Iowa State College Press, Ames., pp 291-328 (1956)].

EXAMPLE 2

EFFECT OF CHRONIC DIETARY TREATMENT WITH TRYPTOPHAN ON THE DEVELOPMENT OF HYPERTENSION, BRAIN CATECHOLAMINES, AND BINDING OF ANGIOTENSIN II TO DIENCEPHALIC TISSUE OF DOCA-TREATED RATS

This experiment was carried out in a fashion identical to that of Example 1 except that during week 10, an assessment of the responsiveness to AII was made by testing the changes in tail skin (T_{sk}) and colonic (T_{co}) temperatures to an acute administration of AII [Wilson et al, In: Angiotensin II-induced Hypothermia in Rats, J. Appl. Physiol., Vol. 58, pp. 534-543 (1985a); Wilson et al, In: Factors Affecting Angiotensin II-induced Hypothermia in Rats, Peptides, Vol. 6, pp. 695-701 (1985b)]. Additional differences between the two experiments occurred at the end of the study when the brain of

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each rat was removed and the diencephalic and mesencephalic portions were separated for analysis of angiotensin II receptors (diencephalic) and for measurement of the content of norepinephrine, dopamine, serotonin, and 5-hydroxyindole acetic acid (mesencephalic), respectively.

Colonic (T_{co}) and tail skin (T_{sk}) temperatures were measured at an ambient temperature of 26 $\pm$ 1°C. while the rats were restrained in Plexiglass tunnel-type cages. T_{co} was measured with a copper-constantan thermocouple inserted 5 cm into the colon of each rat. An additional thermocouple was placed on the dorsal surface at the base of the tail for the measurement of T_{sk}. Both thermocouples were secured to the tail with adhesive tape. The thermocouples were connected to a recording potentiometer which automatically measured the temperature of each thermocouple at 6 minute intervals. Rats were allowed one hour to adjust to the restraining cages after which a 30 minute control period began. During this time, basal T_{co} and T_{sk}measurements were made. At the end of the control period, each rat was injected with AII (100 µg/kg,s.c.) through a slot in the cage. Measurements continued for 90 minutes thereafter.

A detailed description of the binding assay for AII in the diencephalon of the rat is described by [Fregly et al, In: Reduced Dipsogenic Responsiveness to Intracerebroventricularly Administered Angiotensin II in Estrogen-treated Rats, Brain Res., Vol. 338, pp. 115-121 (1985); Wilson et al, In: Mineralocorticoids Modulate Central Angiotensin II Receptors in Rats, Brain Res., Vol. 382, pp. 87-96 (1985)]. The brain was cut anterior to the preoptic region and at the level of the mammillary bodies to represent the anterior and

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posterior limits of the diencephalic block. The lateral limits included the edges of the lateral hypothalamus. The block of tissue (mean weight = 100 mg) included the thalamus, hypothalamus, and septum. Specific areas included the preoptic area, paraventricular nucleus, organum vasculosum of the lamina terminalis, subfornical organ, anterior and posterior nuclei, and dorsomedial and ventromedial nuclei. Protein concentration of the brain particulate fraction was determined by the method of [Lowry et al, In: Protein Measurement with the Folin-Phenol Reagent, J. Biol. Chem., Vol. 193, pp. 265-275 (1951)], and binding data were expressed as fmol/mg protein.

For analysis of catecholamines in brain, the mesencephalon as defined by [Gispen et al, In: Brain RNA and Hypophysectomy; A Topographical Study; Neuroendocrinology, Vol. 9, pp. 285-296 (1972)] was removed, weighed, and homogenized in 1 ml of 0.1 M perchloric acid with glutathione (10 µg) and disodium EDTA (50 µg) added. The homogenate was centrifuged in a refrigerated centrifuge (3,000 x g); the supernatant was removed and filtered using Millipore filters (0.2 µM). The filtrate was assayed directly by HPLC. Chromatographic conditions were similar to those used to analyze urinary catecholamines. All values were standardized to a unit of wet tissue weight.

At death, the heart, right kidney, adrenals, and thyroid gland were removed, cleaned of extraneous tissue, and weighed on a torsion balance.

Statistical analyses of the data were performed as described in Example 1.

From Example 1 and FIG. 1A, it is apparent that chronic dietary administration of tryptophan

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protected against the elevation of blood pressure induced by DOCA. A two-way repeated measures ANOVA revealed a significant effect of treatment ($F=8.55$; $P<0.01$) a significant effect of time ($F=6.35$; $P<0.01$), and a signficant group x time interaction ($F=3.47$; $P<0.01$). The systolic blood pressure of rats treated with DOCA alone increased within 4 weeks of treatment and was elevated significantly ($P<0.01$) above that of the other three groups by five weeks. The significant elevation in the blood pressure of the DOCA-treated group remained for the duration of the experiment. The systolic blood pressures of the groups treated with DOCA and 2.5 and 5.0% tryptophan, respectively, did not differ either from one another or from the control group throughout the experiment, although they differed significantly from the group treated with DOCA alone. Mean body weights of the treated groups did not differ significantly from the control group at any time during the experiment (Figure 1B).

Since Examples 1 and 2 were carried out in an identical fashion with similar results, the data collected under the same conditions have been combined for statistical analysis.

Chronic treatment with DOCA increased significantly ($P<0.01$) the daily intake of isotonic saline while administration of tryptophan to DOCA-treated rats reduced the intake of saline in a dose-related fashion (FIG. 2, left panel). Output of urine paralleled the intake of isotonic saline for each of the four groups (FIG. 2, middle panel). Intakes of food were slightly, but not significantly, reduced in the two groups receiving tryptophan (FIG. 2, right panel). Calculation of the daily intakes of tryptophan added to the diet of the two treated groups revealed that 1.38 and 2.75 g/kg of body weight, respectively, were ingested.

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Administration of DOCA increased daily urinary sodium output significantly ($P<0.05$) while simultaneous treatment with tryptophan reduced sodium output in a graded fashion to control level (Table 1).

Table I

Effect of L-Tryptophan on Daily Urine Output, Urinary Sodium, Potassium, Creatinine and Catecholamine Outputs*

Treatment	Urine Output (ml/kg)	Urinary Output of: Sodium (mEq/kg)	Potassium (mEq/kg)	Creatinine (mg/kg)	Norepi. (ng/mg Creatinine)	Epi. (ng/mg Creatinine)	Dopamine (ng/mg Creatinine)
Control	125.4 ± 24.2•	31.9 ± 5.4	13.0 ± 0.9	30.7 ± 2.7	2.36 ± 0.20	0.46 ± 0.09	12.37 ± 1.40
DOCA	268.2 ± 38.5‡	55.5 ± 8.4‡	13.3 ± 0.9	38.5 ± 1.9‡	2.66 ± 0.25	0.51 ± 0.07	22.16 ± 3.50‡
DOCA + 2.5% Tryptophan	241.4 ± 42.8	51.9 ± 7.0	13.4 ± 0.8	40.5 ± 2.9	2.51 ± 0.09	0.58 ± 0.07	16.83 ± 0.87
DOCA + 5.0% Tryptophan	164.6 ± 37.8	34.2 ± 4.9	12.2 ± 0.7	37.9 ± 4.0	2.12 ± 0.19	0.63 ± 0.07	15.67 ± 1.16

*Performed during week 5 of treatment with tryptophan; data represent means of 3 days of measurements.
•One standard error of mean.
‡Significantly different from control ($P<0.05$).

Chronic administration of DOCA, with or without tryptophan, had no significant effect on urinary potassium output. Urinary creatinine output (mg/day) was increased above the level of the control group in all groups receiving DOCA but was only significantly ($P<0.05$) elevated above control level in the group receiving DOCA alone and DOCA + 2.5% tryptophan. The higher dose of tryptophan returned

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urinary creatinine output to the level of the control group. Chronic treatment with DOCA increased the output of dopamine into urine (ng/mg creatinine) significantly ($P<0.05$) above that of the control group. Simultaneous treatment with tryptophan reduced urinary output of dopamine to levels between that of the DOCA-treated and untreated control groups. Treatment with either or both compounds had no significant effect on urinary outputs of epinephrine and norepinephrine. Similar results were observed when the data were expressed as total ng of each catecholamine excreted per day. A direct linear relationship between daily urinary dopamine output (ng/mg creatinine) and urinary sodium output was observed in the three DOCA-treatment groups (FIG. 3).

Chronic treatment with DOCA increased significantly the dipsogenic responsiveness to acute administration of AII (FIG. 4). Administration of the higher dose of tryptophan reduced the intake of 0.15 M NaCl solution by DOCA-treated rats toward that of the control group. Semi-logarithmic transformation of the data and regression analysis gave the following relationships between intake of 0.15 M NaCl solution (Y) and log-dose of AII (X) for the four groups used in Examples 1 and 2:

Control: $Y = \log 31.7X - 32.1$; $r = 0.59$; $n = 16$; $P = 0.01$

DOCA-treated: $Y = \log 30.6X - 6.9$; $r = 0.48$; $n = 16$; $P<0.05$

DOCA + 2.5% tryptophan: $Y = 26.2X - 1.2$; $r = 0.48$; $n = 16$; $P<0.05$

DOCA + 5.0% tryptophan: $Y = 29.5X - 22.2$; $r = 0.47$; $n = 16$; $p = 0.05$

Statistical comparison of the slopes and intercepts among the four groups revealed significant

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($P<0.01$) differences between the intercepts, but not slopes, of the control group compared with either the DOCA- or DOCA + 2.5% tryptophan treated groups. Neither slopes nor intercepts of the control and DOCA + 5.0% tryptophan-treated groups differed significantly.

Chronic treatment with DOCA, alone or in combination with tryptophan, significantly ($P<0.05$) reduced the response of heart rate to administration of the beta-adrenoceptor agonist, isoproterenol (two way repeated measures ANOVA, $F(2.55)$ between groups = 2.75; $F(\text{time}) = 85.92$; group x time interaction, not significant) (FIG. 5). This analysis was performed on the combined data from Examples 1 and 2. The heart rates of the three groups treated with DOCA did not differ significantly from one another throughout the experiment. The difference between groups was apparently due to treatment with DOCA. The fact that the only significant effect was in the time-course of the response suggests that treatment with DOCA may have influenced the clearance of isoproterenol.

The dipsogenic response to acute administration of isoproterenol was attenuated significantly by chronic administration of DOCA, with or without tryptophan, during the first half-hour after initiation of treatment for the group given DOCA alone and DOCA with the higher dose of tryptophan (Table 2).

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Table 2

Effect of L-Tryptophan on Isoproterenol (5 µg/kg, s.c.)-Induced drinking in DOCA-Treated, Uninephrectomized Rats*

Treatment	Cumulative 0.15 M.NaCl Intake (ml/kg b.w.) during: 0.5	1.0	2.0 hr	Cumulative Urine Output (ml/kg b.w.) during: 0.5	1.0	2.0 hr	Urine Na Output (mEq/kg/ 2 hr)	Urine K Output (mEq/kg/ 2 hr)	Urine Na/K Ratio
Control	17.5 ± 2.1+	23.2 ± 2.4	25.4 ± 2.6	1.0 ± 0.4	2.6 ± 0.8	9.4 ± 1.0	2.4 ± 0.4	0.56 ± 0.10	3.32 ± 0.43
DOCA	8.8 ± 3.4‡	18.2 ± 6.0	21.4 ± 7.1	1.5 ± 0.6	5.9 ± 1.7	13.4 ± 3.7	3.4 ± 0.8	0.89 ± 0.20	3.52 ± 0.37
DOCA + 2.5% Tryptophan	9.5 ± 2.4	13.0 ± 3.2	14.4 ± 3.5	1.6 ± 0.6	5.8 ± 1.7	10.4 ± 2.2	2.1 ± 0.4	0.77 ± 0.08	2.37 ± 0.32
DOCA + 5.0% Tryptophan	7.6 ± 3.6‡	14.6 ± 6.0	18.7 ± 7.5	1.5 ± 0.8	6.0 ± 2.6	15.3 ± 5.3	4.4 ± 1.5	0.82 ± 0.16	4.07 ± 0.98

*Performed during week 9 of treatment with tryptophan.
+One standard error of mean.
‡Significantly different from control ($P<0.05$).

The intake of the group given the lower dose of tryptophan was not different from either the DOCA-treated or control groups. At 1.0 and 2.0 hours after administration of isoproterenol, no significant difference was observed between any two groups. Outputs of both urine and electrolytes into urine failed to show differences among the various groups.

When given a choice between water and isotonic saline to drink, rats treated chronically with DOCA ingested approximately twice as much saline as untreated controls (Table 3). Administration of tryptophan to DOCA-treated rats reduced their NaCl intake toward that of the control group in a

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dose-related fashion. Neither water intake nor the ratio of NaCl/total fluid intake was changed significantly by any treatment. Food intake of the group treated with DOCA + 5.0% tryptophan was increased significantly ($P<0.05$) above that of the control group.

Table 3

Effect of L-Tryptophan on Spontaneous Intakes of Water, 0.15 M. NaCl Solution and Food, in DOCA-treated, Uninephrectomized Rats*

Treatment	No. of Rats	Mean Body Wt. (g)	Mean Intakes (ml or g/100 g b.w./day) of: Food	Water	NaCl soln.	NaCl/Total (%)
Control	16	318 ± 13†	4.4 ± 0.3	2.3 ± 0.3	16.1 ± 1.9	86.2 ± 2.3
DOCA	16	296 ± 7	5.0 ± 0.3	4.4 ± 1.2	29.0 ± 3.5¶	84.3 ± 5.5
DOCA + 2.5% Tryptophan	16	304 ± 8	5.2 ± 0.5	6.9 ± 2.0	24.2 ± 4.0	75.7 ± 7.4
DOCA + 5.0% Tryptophan	16	292 ± 8	6.4 ± 0.8‡	6.5 ± 2.2	17.9 ± 2.6	76.3 ± 5.1

*Performed during week 12 of treatment with tryptophan. ▲ Measurements were carried out over a two day period.
†One standard error of mean.
‡Significantly different from control ($P<0.05$).
¶Significantly different from control ($P<0.01$).

Since a test of renal concentrating ability during a dehydration was carried out in both Examples 1 and 2 at the same time after initiation of treatment with DOCA and tryptophan, the data for each of the four groups in each study were combined and are presented in Table 4.

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Table 4

Effect of Dietary Treatment with L-Tryptophan on Urine Output, Urine Osmolality, and Urinary Sodium and Potassium Output during a 24 Hour Dehydration in DOCA-treated, Uninephrectomized Rats*

Treatment	No. of Rats	Mean Body Wt. (g)	24 Hr. Urine Output (ml/kg)	Urine Osmolality (mOs/kg)	Urinary Na Output (mEq/kg)	Urinary K Output (mEq/kg)	Urine Na/K Ratio
Control	16	299 ± 10+	27.6 ± 1.5	1990 ± 78	6.9 ± 0.3	8.2 ± 0.3	0.84 ± 0.02
DOCA	16	269 ± 5	34.8 ± 3.3‡	1599 ± 98‖	5.7 ± 0.7‡	7.8 ± 0.8	0.73 ± 0.04‖
DOCA + 2.5% Tryptophan	16	280 ± 8	38.2 ± 3.4‡	1546 ± 92‖	6.6 ± 0.5	8.6 ± 0.5	0.77 ± 0.05
DOCA + 5.0% Tryptophan	16	275 ± 8	34.4 ± 2.0‡	1861 ± 132¶	6.1 ± 0.4	9.0 ± 0.6	0.68 ± 0.02‖

*Performed during week 6 of treatment with tryptophan.
+One standard error of mean.
‡Significantly different from Control group (P<0.05).
‖Significantly different from Control group (P<0.01).
¶Significantly different from DOCA-treated group (P<0.05).

During the 24 hour dehydration, treatment with DOCA increased output of urine and decreased its osmolality compared to untreated controls (Table 4). Treatment with the highest dose of tryptophan returned urinary osmolality to the level of controls but had no effect on output of urine. Urinary output of sodium was reduced significantly (P<0.05) from that of the control group by treatment with DOCA while simultaneous treatment with tryptophan returned urinary output of sodium to the level of controls. Urinary output of potassium was unaffected by either treatment. The ratio of Na/K in urine was reduced significantly (P<0.01) from that of controls by

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treatment with DOCA, but was significantly ($P<0.05$) reduced only for the DOCA-treated and DOCA + 5% tryptophan-treated groups. When saline was returned to the rats at the end of the dehydration, fluid intakes of the four groups did not differ signficantly, although a trend to reduce intake of saline was evident in rats treated with graded doses of tryptophan (Table 5).

Table 5

Effect of L-Tryptophan on Intake of Isotonic Saline by DOCA-Salt-Treated Rats after a 24 Hour Dehydration

Treatment	Cum. Saline Intake (ml/kg b.w.) during:			Cum. Urine Output (ml/kg b.w.) during:		
	0.5	1.0	2.0 hr	0.5	1.0	2.0 hr
Control	88.8 ± 4.3*	109.3 ± 4.7	125.5 ± 7.8	9.7 ± 2.0	14.4 ± 2.7	20.4 ± 4.7
DOCA	90.5 ± 7.6	111.5 ± 8.5	137.1 ± 9.8	2.9 ± 1.1†	10.1 ± 1.8	35.0 ± 3.1
DOCA + 2.5% Tryptophan	85.6 ± 6.2	106.2 ± 6.0	129.0 ± 8.2	2.0 ± 0.7†	8.2 ± 1.3	30.0 ± 4.4
DOCA + 5.0% Tryptophan	73.2 ± 6.8	94.2 ± 7.0	114.5 ± 6.6	0.4 ± 0.2†	4.3 ± 1.0†	24.0 ± 3.3

*One standard error of mean.
†Significantly different from control ($P<0.01$).

Outputs of urine during the first half-hour of measurement were reduced significantly ($P<0.01$) by treatment with DOCA, whether or not tryptophan was available simultaneously. There was a trend for a

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decrease in urine output with increasing doses of tryptophan.

Measurement of resting colonic temperature of the rats during the thirteenth week of the experiment showed no significant differences among the groups (Table 6). Resting plasma renin activity was reduced significantly by treatment with DOCA, whether or not tryptophan was also administered. The increased heart weight accompanying treatment with DOCA was reduced by treatment with tryptophan. The increased renal weight induced by DOCA was reduced by tryptophan, but neither to the level of the control group nor in a dose-related fashion. The weight of adrenal glands and thyroid gland were not affected by either treatment.

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Table 6

Effect of L-Tryptophan on Resting Colonic Temperature, Plasma Renin Activity, and Organ/Body Weight Ratio of Certain Organs of DOCA-Treated, Uninephrectomized Rats*

Treatment	No. of Rats	Mean Body Wt. (g)	Resting Colonic Temp. (°C)	Resting PRA (ng/ml/hr)	Organ/Body Wt. (mg/100 g b.w.) Heart	Right Kidney	Adrenals	Thyroid
Control	8	320 ± 12+	37.8 ± 0.5	3.50 ± 0.90	300 ± 9	446 ± 13	18.5 ± 0.9	5.4 ± 0.7
DOCA	8	291 ± 8	38.0 ± 0.4	0.10 ± 0.07¶	360 ± 15‡	561 ± 31‡	18.1 ± 1.4	6.2 ± 0.4
DOCA + 2.5% Tryptophan	8	302 ± 11	37.6 ± 0.4	0.00 ± 0.02¶	333 ± 11	539 ± 35	18.9 ± 1.4	6.7 ± 0.6
DOCA + 5.0% Tryptophan	8	291 ± 9	37.8 ± 0.7	0.15 ± 0.04¶	336 ± 19	533 ± 52	19.8 ± 1.3	6.0 ± 0.5

*Performed during week 13 of treatment with tryptophan.
+One standard error of mean.
‡Significantly different from control ($P<0.05$).
¶Significantly different from control ($P<0.01$).

It can also be seen from the results of Example 2 that chronic dietary treatment with tryptophan protected against the development of DOCA-salt-induced hypertension in this study, as it had in Example 1 (FIG. 6A). There were no significant effects of treatment on body weight throughout the experiment, as was the case in Example 1 (FIG. 6B). Thus, the lower dose of tryptophan appeared to provide protection against the development of hypertension to approximately the same extent as the higher dose.

Acute administration of AII to each group of rats resulted in an increase in the temperature of the

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skin of the tail (T_{sk}) (FIG. 7A). The group treated with DOCA alone had the greatest increase in T_{sk} with significant increases above control level occurring at 25, 30, and 35 minutes after administration of AII. Concurrent administration of tryptophan to DOCA-treated rats reduced the T_{sk} either to (2.5%) or below (5.0%) control level. There were no significant effects of AII on T_{co} when one group was compared with the other at any time during the study although T_{co} of each group decreased from pretreatment level following administration of AII (FIG. 7B). Although there were no significant differences noted among the T_{co} of the four groups during the control period, it is notable that the T_{co} of all groups treated with DOCA were greater than that of the control group.

Measurements of AII-specific binding in the membranes from diencephalic blocks of brain at two levels of AII are shown in FIG. 8. The binding was increased significantly ($P<0.01$) above that of the control group by chronic treatment with DOCA at both levels of AII. Administration of tryptophan to DOCA-treated rats reduced the binding in a graded fashion below that of the control group.

Contents of norepinephrine, dopamine, and serotonin (5-HT) in the mesencephalic portion of the brain were unaffected by treatment with tryptophan (Table 7).

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Table 7

Effect of Chronic Treatment with L-Tryptophan on the Content of Catecholamines and Serotonin in the Mesencephalic Portion of the Brain

Treatment	No. of Rats	Norepi.	Dopamine	Serotonin	HIAA	HIAA/5-HT Ratio
DOCA Control	3	306.6 ± 45.3*	111.3 ± 6.5	670.9 ± 70.8	915.8 ± 21.4	1.4 ± 0.2
DOCA + 2.5% Tryptophan	5	318.0 ± 23.4	115.0 ± 10.1	747.7 ± 117.7	1310.2 ± 73.1	2.1 ± 0.5
DOCA + 5.0% Tryptophan	5	319.7 ± 28.3	117.1 ± 10.1	669.3 ± 51.9	1693.7 ± 110.1	2.6 ± 0.3
One-Way ANOVA						
F-Values:		0.74	0.28	0.33	13.30	3.30
Probability:		>0.05	>0.05	>0.05	<0.01	<0.05

*One standard error of mean.
Values are calculated as ng/g of mesencephalic tissue.

However, the content of 5-hydroxyindole acetic acid (HIAA) in the mesencephalon was increased significantly ($P<0.01$) in rats treated with tryptophan, as was the ratio of HIAA/5-HT ($P<0.05$). This suggests that the turnover of 5-HT in the mesencephalon of the tryptophan-treated groups was increased by dietary supplementation with tryptophan and indicates that peripherally administered tryptophan accessed the brain.

There were no significant changes in resting colonic temperature, in agreement with the data presented in Example 1 (Table 8). At death, the heart and kidneys of the DOCA-treated group were

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significantly increased compared to the control group (Table 8). Treatment with graded doses of tryptophan tended to reduce the weight of the heart to that of the control group. Although the weight of the kidneys was reduced by treatment with tryptophan, it was not returned to the level of the control group. The weight of the thyroid gland was increased by treatment with DOCA but not significantly so. There were no significant effects of treatments on the combined weights of the uterus and ovaries.

Table 8

Effect of L-Tryptophan on Resting Colonic Temperature and Organ/Body Weight Ratio of Certain Organs of DOCA-treated, Uninephrectomized Rats*

Treatment	Resting Colonic Temp (°C.)	Organ/Body Wt. (mg/100 g B.W.) Of: Heart	Kidneys	Uterus + Ovaries	Thyroid
Control	37.4 ± 0.2†	301.3 ± 12.0	455.2 ± 15.8	150.2 ± 22.0	12.0 ± 0.9
DOCA	37.6 ± 0.2	384.3 ± 22.9‡	654.0 ± 43.4‖	136.6 ± 13.4	18.2 ± 3.6
DOCA + 2.5% Tryptophan	37.2 ± 0.2	347.2 ± 16.2	558.3 ± 43.1	127.6 ± 7.3	16.6 ± 2.5
DOCA + 5.0% Tryptophan	37.1 ± 0.3	309.9 ± 16.1¶	571.6 ± 20.1	173.5 ± 25.5	11.0 ± 1.4

*performed during week 16 of the study.
†One standard error of mean.
‡Significantly different from control group ($P<0.05$).
‖Significantly different from control group ($P<0.01$).
¶Significantly different from DOCA-treated group ($P<0.05$).

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The above results show a significant antihypertensive effect of tryptophan in attenuating the elevation of blood pressure, polydipsia, polyuria, salt appetite, and cardiac hypertrophy characteristically accompanying the induction of this type of hypertension. In addition, chronic treatment with tryptophan increased the turnover of serotonin in the mesencephalon of DOCA-treated rats, and attentuated the increased specific binding of angiotensin II to its receptors in the diencephalon. Thus, many potential mechanisms present themselves as possible explanations for the protective effect of treatment with tryptophan. Among these are reduction in the augmented ingestion of NaCl solution, increase in the turnover of serotonin, and reduction of specific AII binding sites in the brain. Other beneficial effects of tryptophan observed here, e.g., improved renal concentrating ability during dehydration and reduced cardiac hypertrophy, are likely to be secondary to the reduction in blood pressure.

EXAMPLE 3

This example demonstrates the antihypertension effect of tryptophan on the renal hypertensive rat. This model of hypertension differs from the DOCA-salt-hypertensive model in a number of respects. Thus, resting plasma renin activity is normal in renal encapsulated rats, but is low in DOCA-salt-induced hypertension. Dissogenic and vascular responses to exogenous administration of AII are increased in the DOCA-treated rats while they are decreased in the renal hypertensive rats. Further, the renal hypertensive rats manifest a salt aversion

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when given a choice between water and isotonic saline solution to drink while the DOCA-treated rats have a salt appetite. Hence, because of these and other differences between the two models of hypertension, it is important to determine whether chronic treatment with tryptophan was as effective an antihypertensive agent in renal hypertensive rats as was observed in the DOCA-treated rats.

Two separate experiments were performed, each of which was similar to Example 1. Both used female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing initially from 180 to 225 g. They were maintained in stainless steel cages and were provided with tap water and finely powdered Purina Laboratory Chow (No. 5001) ad libitum. Infant nursing bottles with cast bronze spouts were used as fluid containers. Food containers were spill-resistant and have been described in detail. The vivarium was maintained at 26 ± 1°C and illuminated from 07.00 to 19.00 h. Unless otherwise desigated, the rats were maintained three per stainless steel suspended cage.

Systolic blood pressures were measured from the tail. A control period preceded initiation of each experiment. During this time, blood pressure and body weight of each rat were measured weekly. At the end of the control period, the rats were divided randomly into the appropriate groups. Bilateral renal encapsulation was performed by the technique of Abrams and Sobin, (Vol. 64, pp. 412-416, 1947) Proc. Soc. exp. Biol. Med., using latex rubber capsules molded to be 1.5-2.0 times the size of the kidneys. All rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) prior to renal encapsulation and were treated with ampicillin daily (5 mg/day, i.m.) for 4 days after renal encapsulation.

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When L-tryptophan was added to food, it was first triturated in a small amount of food using a mortar and pestle. The triturated preparation was then added to finely powdered Purina laboratory chow and mixed in a tumbling mixer at 4 rpm for 1 h.

Statistical analysis of the data was carried out by means of a one-way analysis of variance. The significance of the differences between means of individual groups was assessed by the Newman-Keuls post hoc analysis. Significance was set at the 95% confidence limit.

Experiment 1: Effect of Chronic Dietary Treatment with L-tryptophan on the Development of Renal Hypertension in Rats

Three groups of rats (6 per group) were used. All groups had their kidneys bilaterally encapsulated with latex envelopes. Two of the three groups were given food into which 0.5 and 1.0 g of L-tryptophan was mixed per 100 g of food, respectively. Blood pressure measurements began 1 week after renal encapsulation and continued weekly thereafter for 8 weeks.

During the 4th week of the experiment, the rats were caged individually and water and food intakes measured daily for 5 days. The purpose of this study was to calculate the daily intake of tryptophan by the treated rats and to determine whether the polydipsia characteristic of renal hypertension was influenced by treatment with tryptophan.

At the beginning of the 8th week, resting colonic temperatures of all rats were measured by means of a thermistor recorder using a fast response (15 s) probe. The probe was inserted 5 cm into the colon and held in place for 30 s.

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At the end of the 8th week, the rats were killed by an overdose of pentobarbital. At death, the heart, kidneys, adrenal glands, uterus and thyroid gland were removed, cleaned of extraneous tissue and weighed on a torsion balance to the nearest 0.1 mg.

Experiment 2: Effect of Chronic Dietary Treatment with L-Trytophan on the Development of Renal Hypertension in Rats

Four groups of rats (6 per group) were used. The first 3 groups had their kidneys encapsulated with latex envelopes as described above, while the fourth group underwent a sham operation and served as a normotensive control group. Two of the three renal encapsulated groups were given food into which 2.5 and 5.0 g of tryptophan was thoroughly mixed per 100 g of food, respectively. The remaining group received food without tryptophan supplementation. Measurements of systolic blood pressure began 3 weeks prior to renal encapsulation and continued weekly thereafter for 6 weeks.

During the 3rd week after renal encapsulation the rats were caged individually and water and food intakes and urine outputs were measured daily for 4 days. The urine collected was analyzed for its concentrations of sodium and potassium (flame photometry), creatinine, and catecholamines (norepinephrine, epinephrine, and dopamine) by high-pressure liquid chromatography with electrochemical detection.

During the 5th week, the rats were again caged individually. Water, but not food, was removed from each cage and the animals were allowed to dehydrate for 24 h. Urine was collected under light mineral oil during the 24-hour dehydration. Urinary concentrations of sodium and potassium, as well as

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osmolality (vapor pressure osmometer) were measured. At the end of the dehydration, water was returned to each cage.

During the 7th week of the study, the colonic temperature of each rat was measured as described in experiment 1. The following morning, the rats were killed by decapitation in a room separate from the vivarium. No longer than 10 sec. elapsed between removal of the rat from its cage and decapitation. At death, the heart, kidneys, adrenal glands, and uterus were removed, cleaned of extraneous tissue, and weighed on a torsion balance. The adrenal glands were placed immediately in a freezer at -80°C until analysis for catecholamine content by HPLC with electrochemical detection.

Trunk blood was collected, centrifuged, and serum separated for analysis of its sodium and potassium concentrations. In addition, the concentration of aldosterone in serum was determined by radioimmunoassay.

The results of experiment 1 show that chronic dietary administration of L-tryptophan at concentrations of either 0.5 or 1.0g/100g food failed to prevent the elevation of systolic blood pressure in renal hypertensive rats (FIG. 9A). Treatment did, however, delay the rate of rise of blood pressure without affecting the maximal level attained. Thus, blood pressures of the group receiving 1.0% tryptophan in their diet were significantly below the control level during weeks 1-4 after renal encapsulation. The mean body weight was not affected significantly by the treatment (FIG. 9b).

When food and water intakes were measured during the 4th week after renal encapsulation, no significant differences among the groups were observed

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for food intake, but water intake of the group given 1.0% tryptophan (26.2 ± 2.4g/100g body weight) was increased significantly ($p < 0.05$) above the level of either the control group (17.8 ± 1.6g/100g) or the group given 0.5% tryptophan (19.7 ± 1.3g/100g). The intakes of added tryptophan calculated from the daily food intake were 0.26 and 0.62 g/kg body weight/day for the groups receiving 0.5 and 1.0% tryptophan, respectively.

Resting colonic temperatures measured at the beginning of the 8th week did not differ significantly among the groups. There were also no differences in the organ to body weight ratios of any of the organs removed at death. There was, however, a trend for a reduction in heart/body weight ratio for the group given the higher dose of tryptophan (387.4 ± 44.1 mg/100g body weight) compared to the control group (417.7 ± 52.6 mg/100g) or the group given 0.5% tryptophan (422.9 ± 35.1 mg/100g).

The results of experiment 2 show that chronic dietary administration of tryptophan attenuated the elevation of blood pressure following bilateral renal encapsulation with latex envelopes (FIG. 2a). In this regard, 2.5% appeared to be as potent as 5.0%. There were no effects on body weight resulting from treatment with either dose (FIG. 2b).

Measurements of food and fluid intakes during the 3rd week after treatments began revealed increases in food intake in the tryptophan-treated groups (significant for the group receiving the lower dose) (Table 9). Water intakes of all renal encapsulated groups increased significantly above the level of the control group, without regard to treatment with tryptophan. The increased water intake

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probably reflects the increase in food intake since the ratio of milliliter of water ingested/gram of food ingested remained relatively constant (2.3-2.8) for the four groups. Calculation of the additional intake of tryptophan by the two treated groups, indicated that 1.65 and 3.15 g/kg/day were ingested by the groups given the low and high doses, respectively.

TABLE 9

Effect (mean ± SE) of tryptophan on spontaneous food and fluid intakes of renal encapsulated rats

Treatment	n	Mean body weight, g	Mean intakes	
			food, g/100 BW/day	water, ml/100 g BW/day
Control	6	267 ± 12	4.4 ± 0.4	10.8 ± 0.6
Encapsulation	6	280 ± 8	5.2 ± 0.4	14.4 ± 0.5*
Encapsulation + 2.5% tryptophan	6	277 ± 5	6.6 ± 1.0*	15.0 ± 1.2*
Encapsulation + 5.0% tryptophan	6	273 ± 6	6.3 ± 0.7	16.0 ± 1.2**

The measurements were made over a 4-day period during week 3 of the study.
* Significantly different from control ($p < 0.05$).
** Significantly different from control ($p < 0.01$).

The urine collected during the above study was analyzed for its concentrations of sodium, potassium, creatinine, norepinephrine, epinephrine and dopamine. Outputs of these compounds were calculated from the measured urine output. Renal encapsulated rats excreted significantly more urine than controls, whether or not they were treated with tryptophan (Table 10). There were no differences among groups with respect to urinary outputs of sodium, potassium, creatinine, norepinephrine, and dopamine. There was, however, a significant ($P<0.01$) increase in urinary epinephrine output in the group given the highest dose of tryptophan.

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TABLE 10

. Effect (mean ± SE) of tryptophan on daily urine output and urinary sodium, potassium, creatinine, and catecholamine outputs

Treatment	Urine output (ml/kg)	Urinary output of					
		Na mEq/kg	K mEq/kg	creatinine mg/kg	norepinephrine, ng/mg	epinephrine ng/mg	dopamine ng/mg
Control	65.0 ± 5.0	5.6 ±0.8	12.4 ±1.8	43.4 ±4.3	40.2 ±8.0	15.9 ± 2.4	215.8 ± 37.2
Encapsulation	95.8 ± 3.4[a]	6.7 ±0.4	13.3 ±0.9	42.6 ±0.9	39.0 ±3.6	11.7 ±0.7	213.1 ±22.9
Encapsulation + 2.5% tryptophan	92.1 ± 6.7[a]	7.1 ±0.3	13.1 ±0.7	39.7 ±0.6	39.0 ±3.4	16.2 ± 1.6[c]	255.4 ± 28.0
Encapsulation + 5.0% tryptophan	99.4 ± 5.9[a]	6.7 ±0.5	12.8 ±0.6	36.9 ± 1.2	37.1 ±3.4	91.2 ± 17.4[a, b, c]	249.4 ± 25.5

The measurements were performed during week 3 of the study; data represent means ± SE of 4 days of measurements. Norepinephrine, epinephrine and dopamine output are indicated as ng/mg creatinine.

[a] Significantly different from control ($p < 0.01$).

[b] Significantly different from encapsulation ($p < 0.01$).

[c] Significantly different from encapsulation + 2.5% tryptophan ($p < 0.01$).

When the animals were dehydrated for 24 h during the 5th week of treatment, urine output of the renal encapsulated control group was elevated significantly above that of the control group (Table 11). Treatment with tryptophan reduced urine output toward that of the control group. Similar results were observed when the weight loss during the 24-hour period of dehydration was calculated as a percentage of the initial body weight (Table 11). Osmolality of the urine of the renal encapsulated control group was significantly ($P<0.01$) lower than that of the non-encapsulated control group. Treatment with increasing doses of tryptophan increased osmolality of the urine toward the level of the nonencapsulated control group. Urinary sodium output during dehydration was increased

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significantly by renal encapsulation and returned toward the level of the nonencapsulated control group by treatment with tryptophan. Urinary potassium output was unaffected by any of the treatments.

TABLE 11

Effect (mean ± SE) of 24-hour dehydration on urine output, urine osmolality, body weight, and urinary sodium and potassium outputs in renal encapsulated rats treated with tryptophan

Treatment	Urine output ml/kg	% of initial body weight lost	Urine osmolality (mosm/kg	Urinary output, mEq/kg BW	
				Na	K
Control	29.4 ± 2.4	7.3 ± 0.6	1,485 ± 112	5.1 ± 0.6	7.4 ± 0.6
Encapsulation	64.0 ± 11.5[b]	11.2 ± 1.3[a]	858 ± 129[b]	7.2 ± 0.6[b]	8.6 ± 0.7
Encapsulation + 2.5 % tryptophan	49.6 ± 7.7	10.4 ± 1.0	1,141 ± 171	6.5 ± 0.6	8.8 ± 0.7
Encapsulation + 5.0% tryptophan	42.3 ± 8.1	8.4 ± 0.3	1,538 ± 41[c]	6.3 ± 0.5	9.4 ± 0.6

The measurements were performed during week 5 of the treatment.
[a] Significantly different from control ($p < 0.05$).
[b] Significantly different from control ($p < 0.01$).
[c] Significantly different from encapsulation ($p < 0.05$).

At death, the mean weight of the heart of renal encapsulated rats was increased significantly ($P<0.05$) above that of the control group. Treatment with tryptophan reduced the weight of the heart, but not significantly below that of the untreated, renal encapsulated control group (Table 12). In contrast, the weight of the kidneys of the tryptophan-treated groups were increased significantly above those of either the renal encapsulated group or the controls. There was no significant effect of either renal encapsulation or treatment with tryptophan on weights of adrenals, uterus, serum sodium and potassium concentrations and resting colonic temperature.

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TABLE 12

Effect (mean ± SE) of chronic treatment with tryptophan on organ weights, serum sodium and potassium concentrations and colonic temperature of renal encapsulated rats

Treatment	Organ body weight, mg/100 g BW				Serum, mEq/l		Colonic temperature
	heart	kidneys	adrenals	uterus	Na^+	K^+	°C
Control	282.2 ± 9.0	677.8 ± 37.0	19.6 ± 0.6	132.4 ± 8.3	138.9 ± 2.2	6.8 ± 0.2	37.8 ± 0.2
Encapsulation	335.6 ± 17.7[c]	686.0 ± 23.3	22.9 ± 1.9	124.3 ± 16.5	137.9 ± 1.4	6.8 ± 0.2	37.9 ± 0.2
Encapsulation + 2.5% tryptophan	317.8 ± 5.8[c]	927.2 ± 32.2 [a,b]	21.7 ± 1.0	109.3 ± 4.3	137.8 ± 1.4	6.6 ± 0.1	37.4 ± 0.5
Encapsulation + 5.0% tryptophan	316.9 ± 6.7[c]	858.4 ± 23.9 [a,b]	23.3 ± 1.1	130.9 ± 9.1	140.5 ± 2.2	7.1 ± 0.2	37.9 ± 0.2

The measurements were performed during week 7 of the study.
[a] Significantly different from control ($p < 0.01$).
[b] Significantly different from encapsulation ($p < 0.01$).
[c] Significantly different from control ($p < 0.05$).

Analysis of plasma aldosterone concentration suggests a trend toward an increase with renal encapsulation and a decrease with increasing doses of tryptophan (Table 13). However, the large variability precluded statistical significance. The norepinephrine content (ng/mg of tissue) of the adrenal glands was reduced significantly below the level of the renal encapsulated group, but not the control group, by treatment with tryptophan. However, a dose-related response was not apparent, the contents of epinephrine and dopamine (ng/mg of tissue) in the adrenal glands of the four groups did not differ significantly from one another.

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TABLE 13

Effect (mean ± SE) of chronic administration of tryptophan on adrenal catecholamine content and serum aldosterone levels in renal encapsulated rats

Treatment	Serum aldosterone pg/ml	Adrenal content, ng/mg tissue		
		norepinephrine	epinephrine	dopamine
Control	222.1 ± 64.9[a]	254.1 ± 35.9	963.0 ± 121.6	8.3 ± 1.1
Encap. control	449.2 ± 227.3	311.3 ± 24.2	1,015.1 ± 117.8	8.9 ± 1.2
Encap. + 2.5% trypt.	425.5 ± 175.7	192.9 ± 19.5[b]	899.4 ± 168.9	8.2 ± 0.4
Encap. + 5.0% trypt.	207.1 ± 115.0	199.9 ± 27.2[b]	893.0 ± 68.0	7.1 ± 1.0

The effects were measured during week 7 of the study.

[a] One standard error of mean.

[b] Significantly different from encapsulation control ($p < 0.01$).

The results show that chronic dietary treatment with tryptophan attenuates the elevation of blood pressure in renal hypertensive rats (FIG. 10). These experiments have also established a range of effective doses. Thus, the first experiment in which doses of 0.5 and 1.0% tryptophan were used failed to influence the maximal level of blood pressure attained, but the higher dose (1.0%) did retard the rate of development of the elevated blood presesure (FIG. 1a). In the second study, a concentration of 2.5% tryptophan in the diet appeared to be as effective as 5.0% tryptophan in attenuating the elevation of blood pressure. Thus, a dose between 1.0 and 2.5% appears to be necessary for attentuation of the elevation of blood pressure.

The increased daily water turnover in the renal-encapsulated rats, whether treated with tryptophan or not, may reflect their increased food

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intake (Table 9). The ratio of water/food intake was not different in the four groups. Further, the increased urine output apparently reflects the increased water intake since the ratio of urine output/water intake was similar in the four groups (0.60-0.67). The major striking feature of the analysis of daily urine output was the increased urinary excretion of epinephrine by the group treated with the higher dose of tryptophan (5.0%, Table 10). This suggests a greater rate of secretion of epinephrine by the adrenal medulla which may, in turn, suggest a greater sympathetic outflow in these rats.

Chronic treatment with tryptophan not only attenuated the rise in blood pressure, it also protected against the reduced urinary concentrating ability during a 24-hour dehydration that characteristically accompanies renal encapsulation. A modest (5-6%) effect of treatment to reduce cardiac hypertrophy was also observed. While this effect is small, it must be considered in the context that the extent of cardiac hypertrophy in the untreated renal encapsulated group was about 16% (Table 11).

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EXAMPLE 4

This example demonstrates the anti-hypertensive effects of the chronic administration of L-5-hydroxytryptophan.

Experiment One: Effect of chronic infusion of L-5-hydroxytryptophan on the develop- of DOCA-induced hypertension.

Twenty-four female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing initially from 180 to 225 g were used. They were maintained in stainless steel cages and were provided with tap water and finely powdered Purina Laboratory Chow (5001) ad libitum. Infant nursing bottles with cast aluminum spouts were used as fluid containers (Example 1). Food containers were spill-resistant (Example 1). The vivarium was maintained at 26 ± 1°C and illuminated from 7 a.m. to 7 p.m. Unless otherwise designated, the rats were maintained three per cage.

Systolic blood pressures were measured from the tail using the technique described above and a NarcoBio Instruments Co. polygraph. A two-week control period preceded initiation of this experiment. During this time, blood pressure and body weight of each rat were measured weekly.

At the end of the control period, the rats were divided randomly into four equal groups. Unilateral (left) nephrectomy was then performed on all animals while they were anaesthetized with pentobarbital (40 mg/kg intraperitoneally). At this time, two preweighed 25 mm long silastic tubes (602-265), filled with finely powdered, crystalline DOCA and sealed with silastic cement, were implanted s.c. between the shoulder blades of three of the four groups. The remaining group was implanted s.c. with

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an empty silastic tube. At the end of the experiment, the tubes were removed, dried for 72 h in a desiccator, and weighed on an analytical balance. Based on the change in weight of the tubes and the mean weight of the animals during the time the tubes were implanted, an average of 1.36 ± 0.09 (s.e.) mg/kg per day (297 ± 22 µg/day) were released from the tube.

Immediately after nephrectomy and implantation of silastic tubes, two of the three DOCA-treated groups were implanted s.c. on the left side with Alzet osmotic minipumps (2001, 1 µl/h pumping rate) filled with L-5-HTP at a concentration of 162.5 and 525 mg/ml, respectively. This provided a constant output of 6.3 and 12.6 mg 5-HTP/day (approximately 16.5 and 53.0 µg/kg per day). The pumps are designed to last 7 days, after which replacement is necessary. The remaining DOCA-treated and control groups received an osmotic minipump containing saline. All rats were allowed only 0.15 mol/l NaCl solution to drink. To assure that the 5-HTP remained stable during the 7 days the pumps were implanted s.c., the first set of pumps was removed at 6.5 days. The remaining solution in the pump from each rat in the two 5-HTP treated groups was removed, pooled by group and the concentrations of 5-HTP measured by high performance liquid chromatography (HPLC) with electrochemical detection under the following conditions: the mobile phase consisted of NaH_2PO_4 (50 mmol/l), and CH_3OH (7%). The stable phase was a C_{18} uBondapak column. The flow through the column was 0.6 ml/min and the electrode voltage of the electrochemical detector was 0.65 volts.

A single peak with a retention time identical with authentic 5-HTP was found in the pumps.

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The two concentrations of 5-HTP remaining in the tubes were also identical to those introduced into the pumps prior to implantation. Thus, 5-HTP appears to be stable for at least a week when implanted s.c. in osmotic pumps.

Following unilateral nephrectomy, implantation of tubes containing DOCA and the osmotic minipumps, blood pressures and body weights were measured weekly for 6 weeks.

During the third week, the rats were kept individually in stainless steel metabolic cages. They were provided with their usual diet and 0.15 mol/l NaCl solution to drink. Urine was collected daily for 4 days in Ehrlenmeyer flasks containing 1.0 ml 6 N HCl. Urinary concentrations of norepinephrine, epinephrine, and dopamine were measured by HPLC with electrochemical detection. In brief, urinary catecholamines were isolated on cation resin exchange columns (Biorex 70 cation resin, Biorad Laboratories), eluted with 2 mol/l ammonium sulphate, adsorbed on alumina, and eluted from alumina with 0.1 mol/l perchloric acid. Dihydroxybenzylamine hydrobromide was used as an internal standard. The mobile phase used was a 0.1 mol/l (pH 3.0) monochloroacetate buffer, containing 1 mmol/l sodium EDTA, 600 mg of sodium octyl sulphate and 10% acetonitrile, volume by volume (v/v). The stable phase consisted of a C_{18} uBondapak column. Flow rate was set at 1.2 ml/min and the electrochemical detector at 0.65 volts. All samples collected in one day were extracted and analyzed together and the data normalized to a milligram of creatinine excretion. Urinary creatinine concentration was measured by the method described above while urinary sodium and potassium

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concentrations were measured by flame photometry using lithium as the internal standard.

During the fifth week of the experiment, all rats, maintained in individual metabolic cages, were dehydrated with food available for 24 h. Body weight was measured before and at the end of dehydration. Urine was collected under light mineral oil. Osmolality (by vapor pressure osmometry) and sodium and potassium concentrations (by flame photometry) of the urine excreted by each rat were also measured. At the end of the 24 h period of dehydration, each rat was given a preweighed bottle of isotonic saline (26°C) to drink, and fluid intakes and urine outputs were measured hourly for 2 h.

At the beginning of the seventh week of the experiment, urinary catecholamine excretion was measured during a 2-h exposure to cold stress. Rats were placed in individual metabolic cages in a room maintained at 5 ± 1°C. Immediately prior to exposure to cold, each unanaesthetized rat was injected with distilled water (3% of body weight warmed to 37°C, intraperitoneally). The urine excreted during the 2 h was collected in flasks containing 1.0 ml of 6 N HCl. At the end of the experiment, urine volume was measured and urine was frozen at - 80°C. Colonic temperature was measured prior to loading with water and at the end of the 2-h exposure to cold by means of a Yellow Springs Thermister-Recorder and a fast (15 s) response probe which was inserted 5 cm into the colon and held in place for 30 s. The concentrations of norepinephrine, epinephrine and dopamine in the urine were measured by HPLC as described above. Urinary sodium and potassium concentrations were measured by flame photometry, and urinary creatinine concentration by the method described above.

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At the end of the seventh week, the rats were killed by decapitation and blood from the trunk collected in a beaker. Serum was separated at 5°C by centrifugation (3500 g for 10 min) for determination of aldosterone concentration by radio-immunoassay and sodium and potassium concentrations by flame photometry. At death, heart, right kidney, thyroid, adrenal glands, and uterus and ovaries were removed, cleaned of extraneous tissue, and weighed on a torsion balance.

The brain was removed and cut anterior to the preoptic region and at the level of the mammillary bodies to represent the anterior and posterior limits of the diencephalic block. The lateral limits included the edges of the lateral hypothalamus. The block of tissue (mean weight = 100 mg) included the thalamus, hypothalamus, and septum. Specific areas included the preoptic area, paraventricular nucleus, organum vasculosum of the lamina terminalis, subfornical organ, anterior and posterior nuclei, and dorsomedial and ventromedial nuclei. Membranes from this block of tissue were used in a binding assay for AII as described above. Protein concentration of the brain particulate fraction was determined by the method of Lowry et al, supra, and binding data were expressed as fmol/mg protein.

Statistical analysis of the data was carried out by means of an analysis of variance and a Newman-Keuls post hoc test to determine the difference between any two individual means.

Experiment Two: Effect of chronic infusion of L-5-hydroxytryptophan on Development of DOCA-induced hypertension

A second experiment similar to that described in experiment one was carried out. There

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were, however, several differences from experiment one. These included the use of a dose of L-5-HTP lower than those used in experiment one (4.2 mg 5-HPT/day) and one intermediate between the two doses used in experiment one (8.4 mg 5-HTP/day). In addition, only three groups (six rats/groups) were used, i.e., a DOCA-treated control group and two DOCA-treated groups receiving 4.2 and 8.4 mg 5-HTP/day, respectively. Treatment was carried out for 7 weeks.

During the third and seventh weeks of the experiment, all rats were weighed, caged individually without food, and administered 50 and 100 g AII/kg, s.c., respectively. Each rat was then provided with a preweighed bottle of 0.15 mol/l NaCl solution and intake measured thereafter for 1 h.

During the sixth week of the experiment, each rat was administered a load of isotonic saline (3% of body weight, intraperitoneally), warmed to body temperature (37°C), and placed alone into a metabolic cage. Urine was collected during the subsequent 4 h and the concentrations of sodium and potassium were determined by flame photometry.

The experiment was terminated after the seventh week of treatment. The rats were not killed at the end of this experiment.

Experiment one shows that chronic treatment with 5-HTP at either 6.3 or 12.6 mg/day provided significant protection against the elevation of blood pressure in DOCA-salt-treated rats (FIG. 11a). Blood pressures of the groups treated with 5-HTP remained essentially at the level of the control group throughout the experiment. The gain in body weight was unaffected by treatment with either DOCA or DOCA in combination with the two doses of 5-HTP FIG. 11b).

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Spontaneous intakes of 0.15 mol/l NaCl solution and food were measured for 4 days during the third week of the study. There were no significant differences in either mean body weight or food intakes of the four groups at this time. Intake of NaCl solution by the group receiving DOCA + 6.3 mg 5-HTP/day [53.8 ± 4.6 (s.e.) ml/100 g body weight per day] was significantly ($P<0.01$) greater than in any of the other three groups (control, 32.0 ± 2.0; DOCA, 42.1 ± 6.4, and DOCA + 12.6 mg 5-HTP/day, 29.2 ± 2.5). The intake of the group receiving 12.6 mg 5-HTP/day was essentially at the level of the control group. Output of urine reflected the intake of 0.15 mol/l NaCl, as did output of sodium into urine (Table 14). Output of creatinine into urine was not affected significantly by treatment either with DOCA or with DOCA in combination with either dose of 5-HTP. However, urinary outputs of epinephrine by both groups treated with 5-HTP were elevated significantly above those of both the control and DOCA-treated groups. Urinary output of dopamine was increased significantly above that of the control group by treatment with DOCA and was returned toward the level of controls by treatment with 5-HTP.

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TABLE 14

Effect of chronic administration of L-5-HTP on mean daily outputs of urine, sodium (Na), potassium (K), creatinine and catecholamine by DOA-salt-treated rats*

	Urinary Output						
Treatment	Urine output (ml/kg)	Na (mol/l per kg	K	CR (mg/kg)	N	E (ng/mg CR)	DA
Control	205 ± 21†	45.8 ± 3.4	13.6 ± 0.9	41.1 ± 0.4	104 ± 3	28 ± 2	396 ± 32
DOCA	301 ± 57	60.5 ± 9.4	15.3 ± 2.3	46.3 ± 0.6‡	115 ± 9	26 ± 3	520 ± 37‡
DOCA + 6.3 mg 5-HTP/day	418 ± 40§	81.5 ± 6.6	16.6 ± 0.8	47.4 ± 1.0§	118 ± 22	35 ± 4	467 ± 45
DOCA + 12.6 mg 5-HTP/day	208 ± 20	44.1 ± 4.1	22.9 ± 5.8	45.3 ± 2.1	129 ± 15	36 ± 2	478 ± 40

* Measurements made for 4 days during week three of the experiment. †One standard error of mean. ‡Significantly different from control group ($P<0.05$); §Significantly different from control group ($P<0.01$). Significantly different from DOCA-treated group ($P<0.05$). Abbreviations: DOCA, deoxycorticosterone acetate; CR, creatinine; N, norepinephrine; E, epinephrine; DA, dopamine; HTP, hydroxytryptophan.

The effect of chronic administration of 5-HTP and DOCA on output of urine, urinary osmolality, and urinary sodium and potassium outputs during a 24-h dehydration are shown in Table 15. The output of urine during the dehydration was significantly ($P<0.01$) elevated above the level of the control group by treatment with DOCA. Simultaneous treatment with the higher dose of 5-HTP reduced the output of urine to the level of the control group. Only the osmolality of the urine of the group treated with DOCA + 6.3 mg 5-HTP/day was reduced significantly below the level of the control group, although all groups treated with DOCA had osmolalities that were lower than those of the control on average. There was no sigificant difference between any two groups with respect to urinary output of sodium, but urinary output of potassium by the DOCA-treated group was increased significantly ($P<0.05$) above that of the control group. When 5-HTP was given in combination with DOCA, output of potassium was returned to control level by treatment with the higher dose. The ratio of sodium/potassium in urine was reduced significantly below the level of the control group by treatment with DOCA and returned to the level of the control group by treatment with the lower dose of 5-HTP. The higher dose of 5-HTP had a ratio between that of the DOCA-treated group and the control group.

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TABLE 15

Effect of chronic administration of L-5-HTP on urine output, urine osmolality and urinary sodium (Na) and potassium (K) outputs during a 24-h period of dehydration in DOCA-treated, uninephrectomized rats*

Treatment	No. of rats	Mean body weight (g)	24-h Urine output (mg/kg)	Urine osmality (mg/kg)	Urinary output mmol/l per kg per 2 h Na	K	Na/K ratio
Control	6	267 ± 8†	23.9 ± 4.3	2114 ± 350	5.8 ± 0.4	6.6 ± 0.5	0.89 ± 0.06
DOCA	6	261 ± 10	38.7 ± 1.5‡	1501 ± 111	6.3 ± 0.5	9.8 ± 0.8‡	0.65 ± 0.06‡
DOCA + 6.3 mg 5-HTP/day	6	262 ± 10	37.9 ± 1.6‡	1195 ± 55‡	6.0 ± 0.4	7.1 ± 0.8	0.87 ± 0.05
DOCA + 12.6 mg 5-HTP/day	6	265 ± 7	26.6 ± 3.6	1682 ± 131	5.3 ± 0.9	6.8 ± 1.3	0.77 ± 0.02

* Performed during week five of the experiment. †One standard error of the mean.
‡Significantly different from control group ($P<0.05$); §significantly different from control group ($P<0.01$); Significantly different from DOCA-treated group ($P<0.05$).
Abbreviations: DOCA, deoxycorticosterone acetate; HTP, hydroxytryptophan.

There were no significant differences among the four groups with respect to the effect of a 2-h exposure to cold (5°C) on output of urine, sodium, potassium, creatinine, norepinephrine, or dopamine. In contrast, all three groups treated with DOCA had twice as much epinephrine in their urine as the control group [control, 27 ± 4 (s.e.); DOCA, 61 ± 13; DOCA + 6.3 (5-HTP/day, 52 ± 7; DOCA + 12.6 mg 5-HTP/day, 50 ± 7 ng/mg creatinine]. Treatment with 5-HTP did not significantly alter the elevation in epinephrine output induced in DOCA-treated rats by acute exposure to cold. Mean colonic temperatures of the four groups did not differ from one another either before or after the 2-h exposure to cold. However, the colonic temperatures after removal from cold were approximately one degree centigrade lower than pre-exposure values for each group.

At the end of the experiment (week 7), the rats were killed and the brains removed. The specific binding of AII to its receptors in membranes from cells of the diencephalon showed that a significant ($P<0.01$) increase above that of the control group occurred in the group treated with DOCA (FIG. 12). Treatment with 5-HTP reduced the specific binding toward that of the control group. The specific binding of AII by the two 5-HTP treated groups did not differ from one another, nor did they differ significantly from the control group. They were, however, significantly ($P<0.01$) different from the DOCA-treated group.

At death, the mean weight of the heart of the DOCA-treated group was significantly larger than that of the control group (Table 16). Treatment with 5-HTP reduced the weight of the heart to that of the

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control group. On average, the weight of the right kidney appeared to increase during treatment with DOCA and to return toward the level of controls during treatment with 5-HTP. Differences, however, were not significant. There was no significant effect of the two treatments on weight of adrenal glands, uterus plus ovaries, and thyroid gland.

Measurements of sodium and potassium concentrations in plasma of rats revealed no significant differences between any two groups for these two elements (Table 16). There was, however a significant ($P<0.01$) reduction below control level in the concentration of aldosterone in plasma of all three groups treated with DOCA [control, 203 39 (s.e.); DOCA, 66 ± 20; DOCA + 6.3 mg 5-HTP/day, 87 ± 25; DOCA + 12.6 mg 5-HTP/day, 74 ± 12 pg/ml]. Additional treatment with 5-HTP had no further effects on plasma aldosterone concentration.

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TABLE 16

Effect of chronic administration of L-5-HTP
on the ratio of organ weight to body weight
of certain organs of rats treated chronically with DOCA-salt*

Treatment	Mean body weight (g)	Organ body weight ratio (mg/100 g body weight)				
		Heart	Right Kidney	Adrenals	Uterus + ovaries	Thyroid
Control	283 ± 8†	383 ± 12	650 ± 77	24.7 ± 0.6	167 ± 19	7.3 ± 0.5
DOCA	278 ± 7	452 ± 18±	792 ± 64	27.4 ± 3.1	158 ± 7	7.4 ± 0.5
DOCA + 6.3 mg 5-HTP/day	291 ± 9	429 ± 13	746 ± 18	22.5 ± 2.4	109 ± 37	7.5 ± 0.5
DOCA + 12.6 mg 5-HTP/day	280 ± 6	386 ± 27§	697 ± 12	24.0 ± 1.9	128 ± 6	6.9 ± 0.4

* Performed during week of experiment. †One standard error or mean. ±Significantly different from control group ($P<0.01$); §Significantly different from DOCA-treated group ($P<0.01$).
Abbreviations: DOCA, deoxycorticosterone acetate; HTP, hydroxytryptophan.

Experiment two shows that chronic treatment with 5-HTP at doses both lower (4.2 mg/day) and intermediate between (8.2 mg/day) those used in experiment one prevented the elevation of blood pressure in DOCA-treated rats (FIG. 13a). Blood pressures of the groups treated with 5-HTP remained approximately at their pretreatment, control level. Body weight was unaffected by treatment with either dose of 5-HTP (FIG. 13b).

The drinking response to administration of AII (50 and 100 µg/kg, s.c.) was influenced by both the dose of 5-HTP and AII (FIG. 14). Administration of the higher dose of 5-HTP reduced the drinking response to either dose of AII to levels significantly ($P<0.05$) below that of the DOCA-treated control group.

The effect of treatment with the higher dose of 5-HTP on the ability to excrete a load of isotonic saline was to increase urinary output of sodium and potassium significantly ($P<0.01$) above that of the DOCA-treated control group (Table 17). Urine output of the group receiving the higher dose of 5-HTP, while higher than that of the DOCA-treated control group, was not increased significantly.

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TABLE 17

Effect of chronic administration of L-5-HTP on the excretion of a saline load (3% body weight, intraperitoneally) by DOCA-treated, uninephrectomized rats*

Treatment	No. of rats	Mean body weight (g)	Cumulative Urine output (ml/kg per 4 h)	Urinary excretion (mmol/l per kg per 4 h)	
				Sodium	Potassium
DOCA	6	303 ± 8 †	20.0 ± 2.6	10.3 ± 1.3	3.7 ± 0.3
DOCA + 4.2 mg L-5-HTP/day	6	291 ± 6	17.8 ± 2.4	6.2 ± 1.3	2.2 ± 0.5
DOCA + 8.4 mg L-5-HTP/day	6	300 ± 10	26.8 ± 2.9	19.2 ± 3.0 ‡	5.0 ± 0.7 §

* Performed during week seven of the experiment. † One standard error of mean. ‡ Significantly different from DOCA + 4.2 mg L-5-HTP/day ($P<0.05$); § Significantly different from DOCA ($P<0.01$); Abbreviations: DOCA, deoxycorticosterone acetate; HTP, hydroxytryptophan.

The above results clearly show that 5-HTP protects against the development of DOCA-salt-induced hypertension to approximately the same extent as tryptophan. This includes protection against: elevation of blood pressure, cardiac hypertrophy, reduced renal function, increased NaCl intake, and increased binding of AII to sites in the brain. The results of these studies suggest that a dose of 4.2 mg 5-HTP/day can prevent the development of DOCA-induced hypertension.

Chronic administration of 5-HTP attenuated the DOCA-induced increase in specific-binding of AII to its receptors in membranes from the diencephalon of the brain, as well as the disogenic responsiveness to administration of AII.

Chronic administration of 5-HTP at doses as high as 12.6 mg/day had no significant effects on either food intake or body weight. Thus, the compound appears to have no significant toxicity with respect to these parameters.

During the stress of a 2-h exposure to air at 5°C, urinary outputs of norepinephrine and dopamine did not differ among the four groups, but urinary output of epinephrine increased in all DOCA-treated groups. Treatment with 5-HTP had no additional effect. This suggests a greater adrenal medullary response to a given stress in DOCA-treated rats.

EXAMPLE 5

In addition to the studies described above in which hypertensive rats were treated with tryptophan, the effect of chronic treatment with l-tryptophan on the blood pressure of 17 human patients with mild to moderate essential hypertension was also studied.

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Those participating in this 8 week study were patients whose hypertension was untreated when they were referred to the clinic and whose diastolic blood pressure was greater than 90 mm Hg, but less than 110 mm Hg. The blood pressures of these patients were measured weekly until diastolic pressure was constant within a range of 10 mm Hg (usually within 4 weeks) (Control period). A second group of patients was also studied. These consisted of patients on medication whose hypertension was poorly controlled; i.e., diastolic blood pressure was greater than 95 but less than 105 mm Hg. In this group of patients, all medication was stopped and they were observed at weekly intervals until their diastolic blood pressures stabilized within a 10 mm Hg range (usually within 4 weeks). Upon admission to the study, medical histories and physical examinations were carried out on each of these patients. Blood pressures were measured in the supine position after 20 minutes of rest in a dimly lighted room by means of a mercury column sphygmomanometer. The fifth Karothoff sound was used as an approximation of diastolic pressure. In addition, a chest X-ray, a liver function test, complete hemogram (including hematocrit, hemoglobin, red and white blood cell counts, differential blood cell count), urinalysis, plasma creatinine concentration, creatinine clearance and a Zung Depression Test were carried out on each patient.

At the end of the control period, treatment with l-tryptophan began. The dosage and schedule during the first two weeks of treatment varied among the patients beginning with 0.5 g TID (1.5 g/day) in 3 patients and 0.5 g TID + hs (2.0 g/day) in 11 patients while 1 patient received 1.0 g TID (3.0 g/day) and two others received 1.0 g TID + hs (4.0 g/day). During the third through fifth weeks, 4 patients received 0.5 g TID + hs; 6 received 1.0 g TID, while the

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remaining 6 received 1.0 g TID + hs. During the sixth through eighth weeks, 4 patients received 0.5 g TID + hs while the remaining 13 patients received 1.0 g TID + hs. Each patient was seen at weekly intervals for 3 weeks to ascertain the effectiveness of the medications. Another group of 25 patients served as controls and received a placebo for 4 weeks.

The results of this study show that the reclining blood pressure of the placebo-treated controls remained elevated throughout the 5 weeks (one control week and four weeks of the experimental period) that measurements were made (FIG. 15). On the other hand, both reclining systolic and diastolic blood pressure of J.P. (FIG. 16) decreased during the course of treatment with increasing doses of tryptophan. When a regression of reclining blood pressure (systolic and diastolic) versus dosage of tryptophan (g/day) administered was calculated, there was a significant ($P<0.05$, systolic; $P<0.01$, diastolic) correlation, with a negative slope for both diastolic and systolic blood pressure (FIG. 17). This indicates that blood pressure declined as dosage of tryptophan increased, with 4.0 g/day yielding the best response. A similar response of blood pressure to administration of tryptophan occurred in patient M.D. (FIG. 18). This patient was followed for 4 additional weeks after treatment with tryptophan ceased and no other medication was given. The average blood pressure during these four weeks is shown as the last point in the figure and is designated "C" on the abscissa (FIG. 18). Both diastolic and systolic blood pressures increased in the absence of treatment indicating that tryptophan exerted an antihypertensive effect. A regression of blood pressure versus dosage of tryptophan administered is shown for this patient (M.D.) in FIG. 19. There is again a significant ($P<0.01$, systolic; $P<0.05$, diastolic) correlation

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between both blood pressures and daily dose of tryptophan administered. Again, the slopes of the regressions were negative, indicating that blood pressure declined as dose of tryptophan increased. These data also show that 4.0 g of tryptophan daily gave the best response.

A slightly different response was observed in patient, R.C. (FIG. 20). This patient received only two doses of tryptophan (2.0 and 4.0 g/day). As observed in FIG. 20, 2.0 g/day had no effect on blood pressure. When treated with 4.0 g/day (beginning week 3), diastolic blood pressure declined. Again, when treatment ceased and no other drugs were administered, the average blood pressure for the four weeks off drugs increased ("C" on abscissa of FIG. 20), indicating that tryptophan exerted an antihypertensive effect. The regression of blood pressure versus dose of tryptophan (g/day) (FIG. 21) reveals a significant ($P<0.01$) correlation between diastolic blood pressure and daily dose of tryptophan. In contrast, systolic blood pressure was unaffected. Since the diastolic blood pressure reflects peripheral vascular resistance (an increase of which is the primary cause of hypertension), the significant decline in diastolic blood pressure with increasing dose of tryptophan is physiologically and medically significant since it signifies a reduction in peripheral vascular resistance.

Although the data for only three of the 17 treated patients are given for brevity of discussion, it can be stated that 9 of the 16 patients treated with tryptophan had a significant ($P<0.05-<0.01$) decrease in mean arterial blood pressure during the 8 weeks of treatment. One patient was dropped from the study during the sixth week because of non-compliance with therapy. Two additional patients were partially responsive [correlation coefficients of the relation-

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ship between blood pressure (systolic and diastolic) versus dose of tryptophan = 0.40 to 0.60] and six (correlation coefficients = 0.10 to 0.39), including the non-compliant patient, were unresponsive.

There were no significant adverse effects of therapy. Liver function tests (including SGOT, SGPT, alkaline phosphatase, total and indirect bilirubin and serum albumin) during treatment were unchanged from those made prior to treatment in all patients. Comparison of chest X-rays taken before administration of tryptophan with those taken after 7 to 8 weeks of treatment showed no change in either lungs or heart. Routine urine analysis, plasma creatinine concentration and creatinine clearance were also unaffected by treatment with tryptophan. Body weight was not affected significantly by treatment.

The change in reclining systolic and diastolic blood pressure from the control period during the first week of treatment is graphed against the daily dose of tryptophan administered in FIG. 22. The reduction in systolic blood presssure was linear with dose of tryptophan administered, with a maximal reduction of 14 mm Hg occurring at the highest dose (4.0 g/day). The maximal reduction in diastolic blood pressure (-4 mm Hg) also occurred when the highest dose was administered. There were, however, only two patients in the group receiving the highest dose. Both the systolic and diastolic blood pressures of control subjects who received only a placebo decreased by 3 mm Hg during the first week. It thus appears to be clear that tryptophan produced its best response at 4.0 g/day and that the reduction in systolic pressure is greater at this dose of tryptophan than the reduction in diastolic pressure. The greater reduction in systolic than diastolic pressure during the first week of treatment is typical of most effective antihypertensive drugs.

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The change in reclining systolic blood pressure of all tryptophan-treated patients, regardless of dose, at the end of the fourth week of treatment, was -9.4 mm Hg compared to pre-treatment control measurements, while placebo-treated controls had a change of -2.1 mm Hg. In the case of reclining diastolic blood pressure, the tryptophan-treated group had a change from pre-treatment control measurements of -5.4 mm Hg while placebo-treated controls had a change of -1.3 mm Hg. These data again show the effectiveness of tryptophan in reducing the elevated blood pressure of patients with mild to moderate essential hypertension. For more severe types of hypertension, tryptophan may be used alone, or in combination with other antihypertensive therapies, including, among others, thiazide diuretics, ganglionic blockers, angiotensin converting enzyme inhibitors, beta-adrenergic blockers, as well as alpha-1 adrenergic antagonists and alpha-2 adrenergic agonists.

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CLAIMS:

1. A method for the treatment of hypertension in a human patient or non-human animal requiring such treatment consisting essentially of chronically administering to said patient or animal a daily dosage of from about 4 to about 10 grams/day of a member selected from the group consisting of L-tryptophan, L-5-hydroxytryptophan, a pharmaceutically acceptable salt or mixtures thereof for a time sufficient to significantly lower the blood pressure in said animal.

2. The method of claim 1 wherein a daily dosage of from about 4 to about 6 grams/day of said member are administered to said patient or animal.

3. The method of claim 1 for treating a human.

4. The method of claim 1 wherein said daily dosage is administered as a dietary supplement.

5. The method of claim 4 wherein said daily dosage is divided substantially equally among daily feedings.

6. The method of claim 1 wherein said daily dosage is administered orally or parenterally.

7. A method according to claim 1 continued for a time sufficient to maintain blood pressures at predetermined levels.

8. A method for the treatment of a condition selected from the group consisting of cardiac or renal hypertrophy, polydipsia, polyuria, stroke or athersclerosis in a human or non-human animal requiring such treatment consisting essentially of chronically administering to said patient or animal a daily dosage of from about 4 to about 10 grams/day of a member selected from the group consisting of L-tryptophan, L-5-hydroxytryptophan, a pharmaceutically acceptable salt or mixtures thereof for a time sufficient to ameliorate said condition.

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9. The method of claim 8 wherein a daily dosage of from about 4 to about 6 grams/day of said member are administered to said patient or animal.

10. The method of claim 8 for treating a human.

11. The method of claim 8 wherein said daily dosage is administered as a dietary supplement.

12. The method of claim 11 wherein said daily dosage is divided substantially equally among daily feedings.

13. The method of claim 8 wherein said daily dosage is administered orally or parenterally.

14. A method according to claim 8 continued for a time sufficient to continue amelioration of said condition for a predetermined time.

15. A pharmaceutical composition in unit dosage form adapted for the chronic administration to a human patient or non-human animal in need of treatment of hypertension, cardiac or renal hypertrophy, polydipsia, polyuria, stroke or atherosclerosis consisting essentially of an amount of a member selected from the group consisting of L-tryptophan, L-5-hydroxytryptophan, a pharmaceutically acceptable salt or mixtures thereof such that the administration of said composition to said patient or animal will comprise a total daily dosage of from about 4 to about 10 grams/day of said member and a pharmaceutically acceptable carrier therefor.

16. A dietary supplement composition in unit dosage form adapted for the chronic administration to a human or non-human animal in need of treatment of hypertension, cardiac or renal hypertrophy, polydipsia, polyuria, stroke or atherosclerosis consisting essentially of an amount of a member selected from the group consisting of L-tryptophan, L-5-hydroxytryptophan, a pharmaceutially acceptable salt or mixtures thereof such that the administration of said composition to said patient or animal will comprise a total daily dosage of from about 4 to about 10 grams/day of said member and an acceptable dietary carrier therefor.

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FIG. 1A.

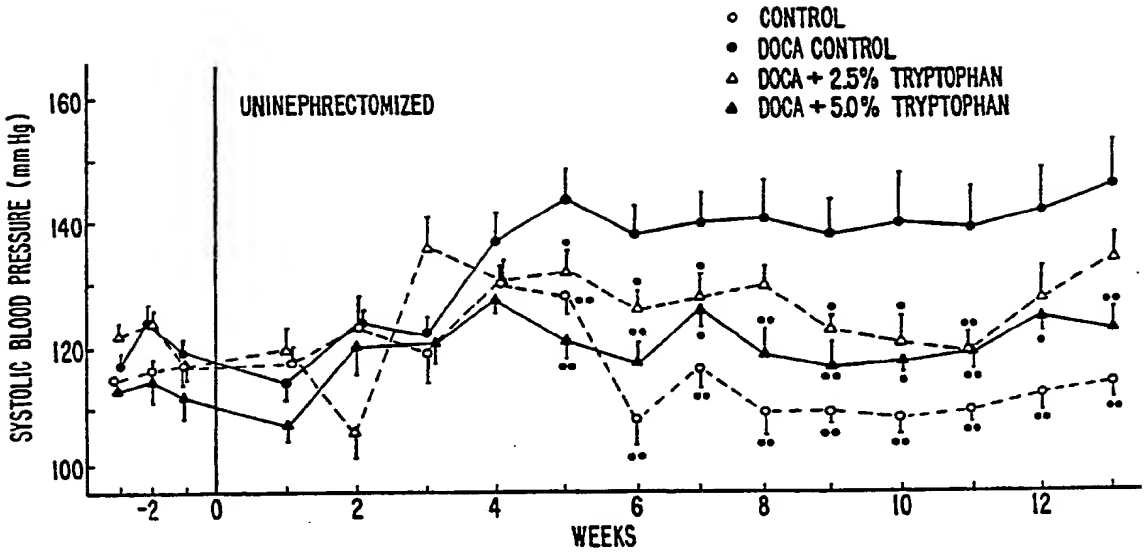

FIG. 1B.

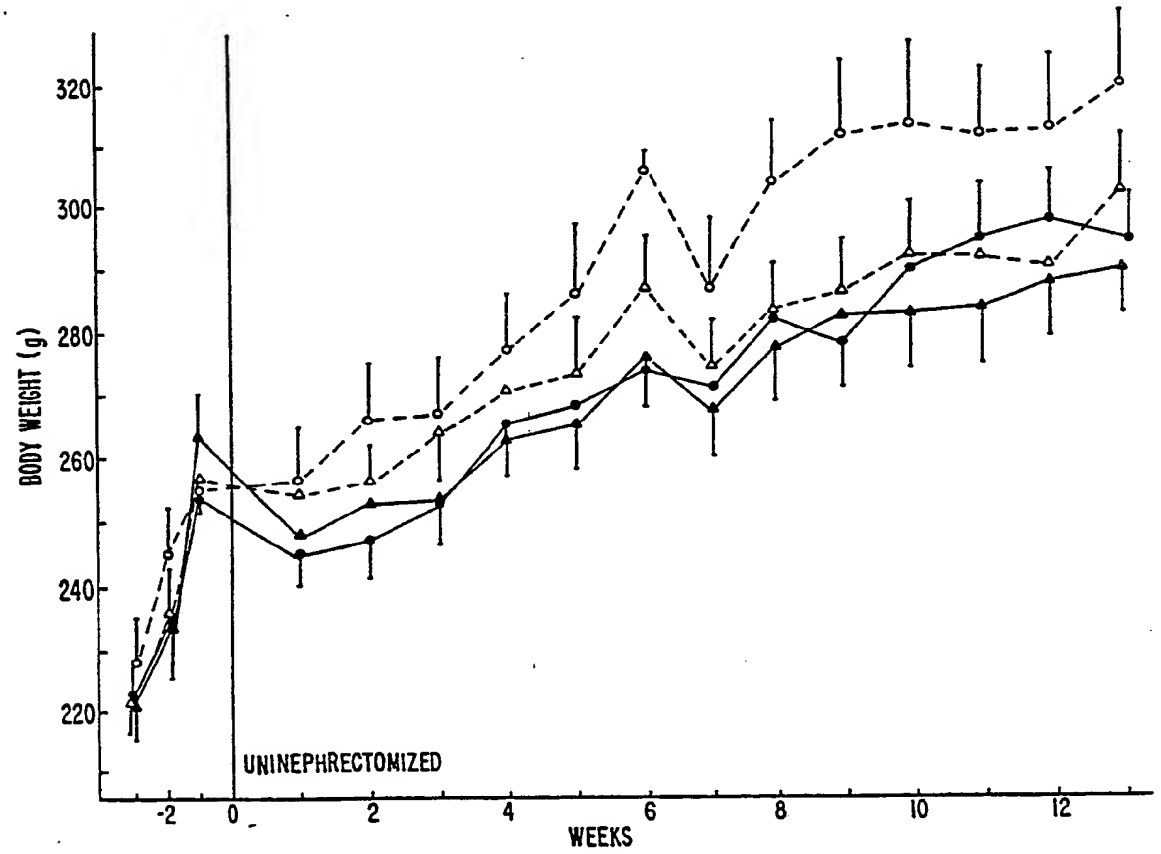

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FIG. 2.

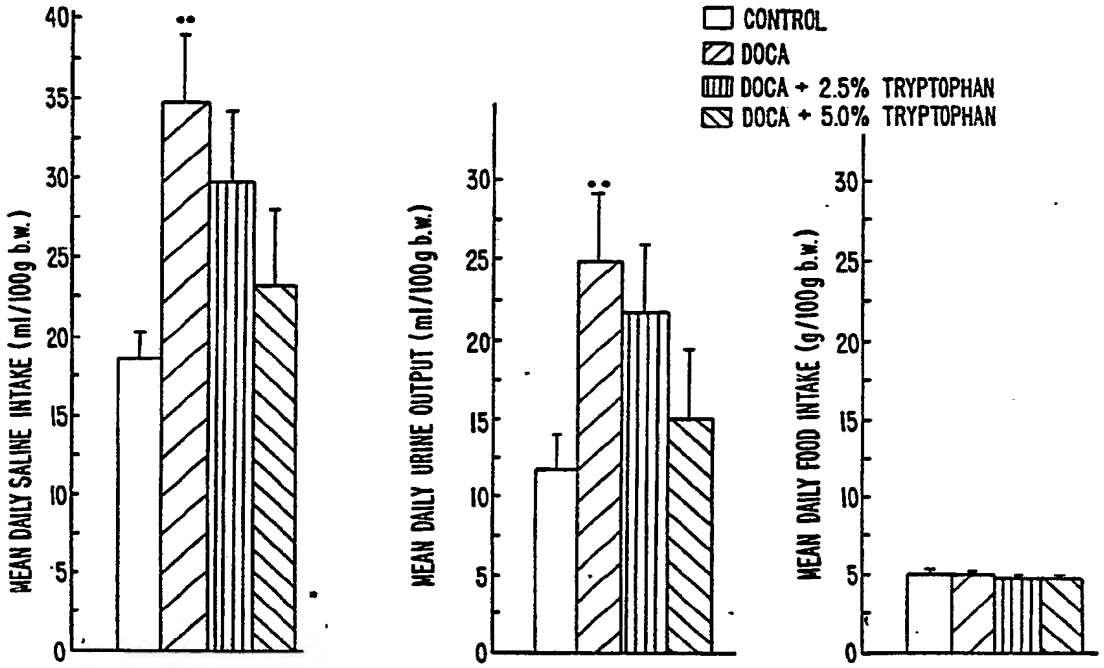

FIG. 3.

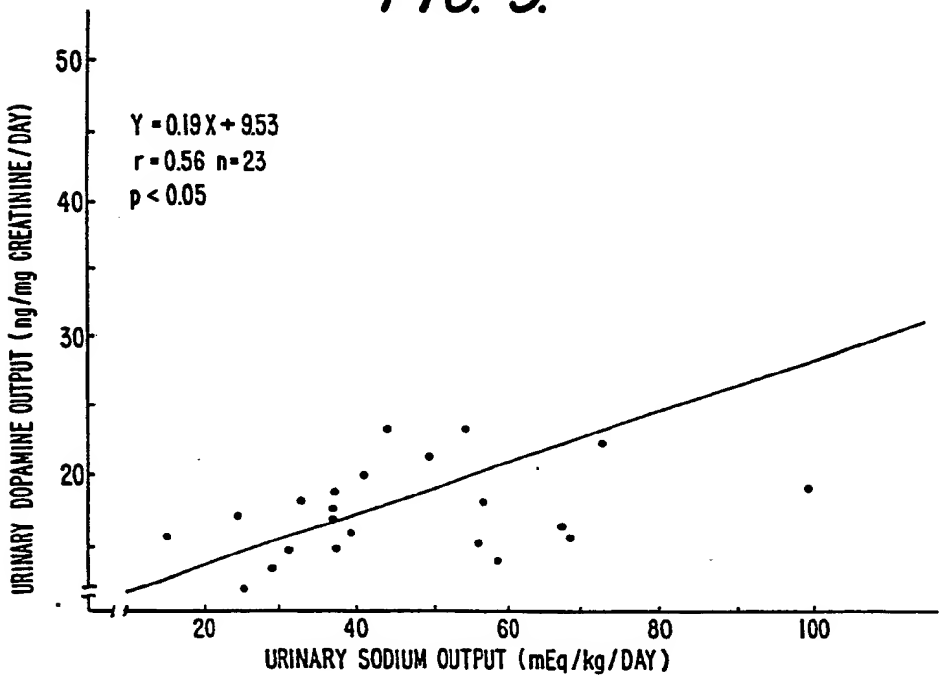

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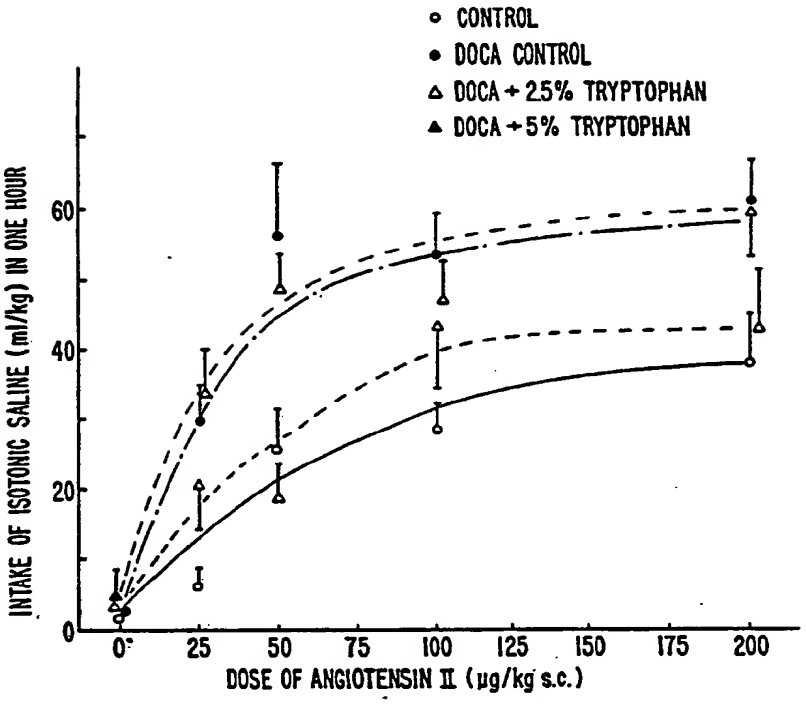
FIG. 4.



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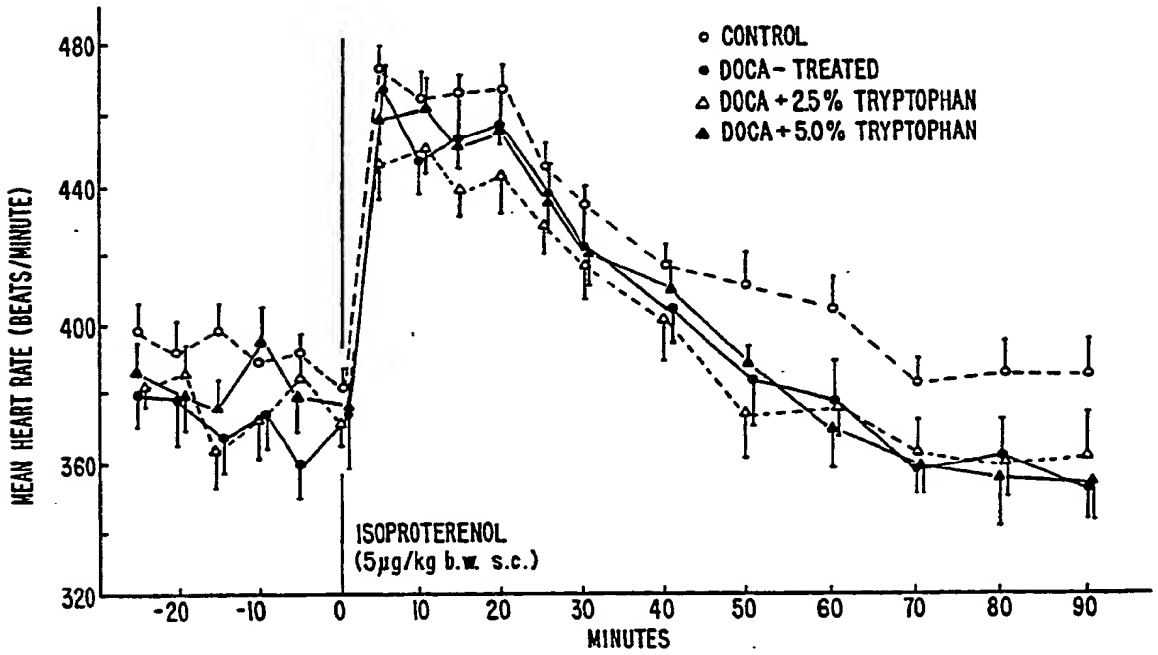

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FIG. 6A.

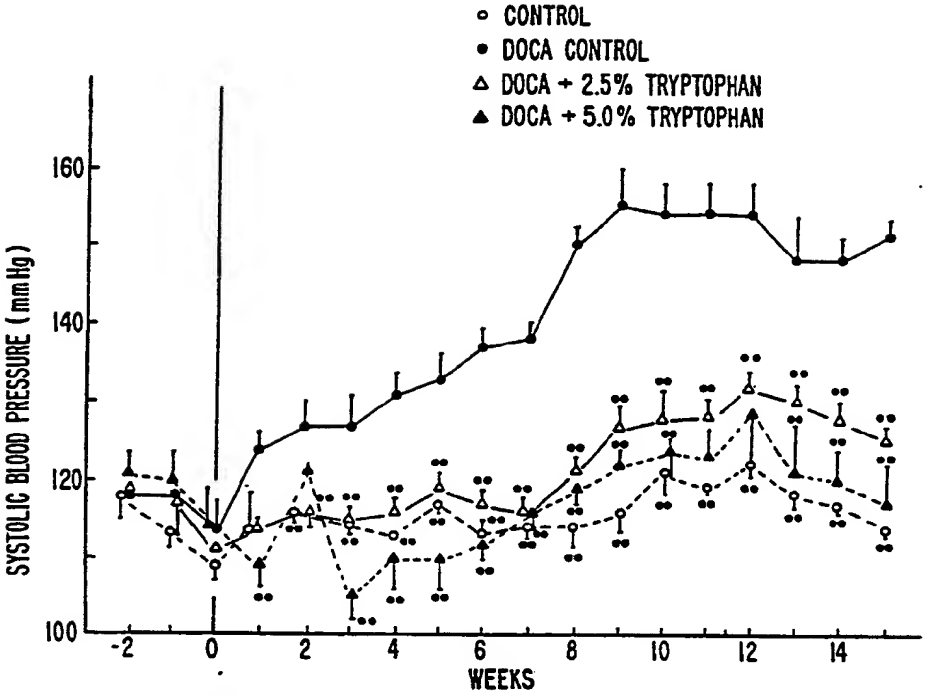

FIG. 6B.

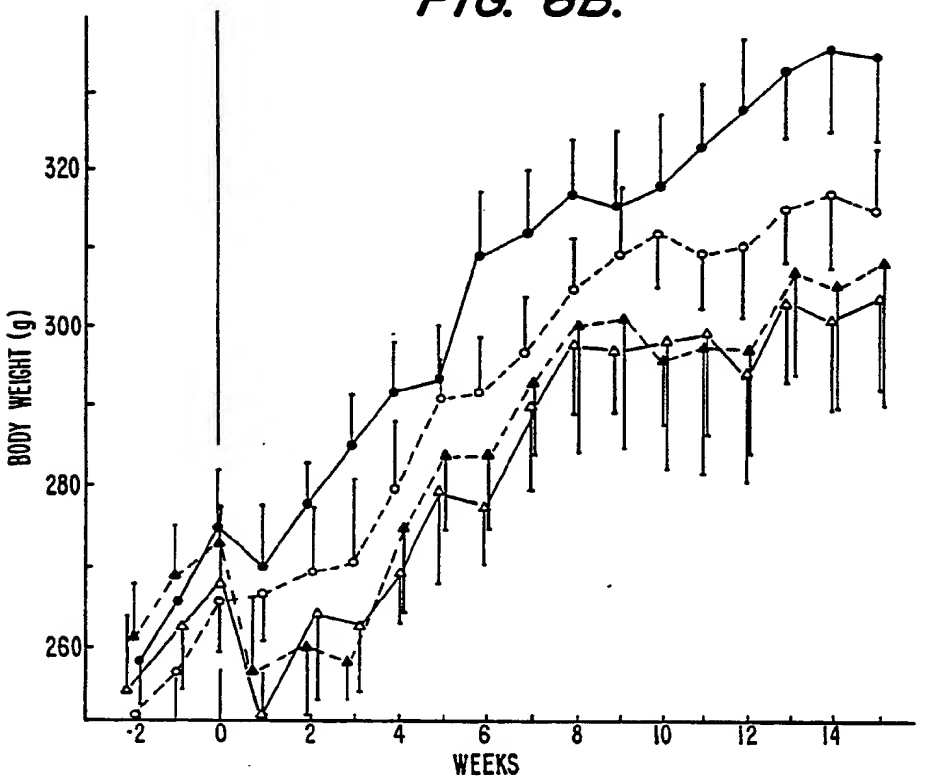

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FIG. 7A.

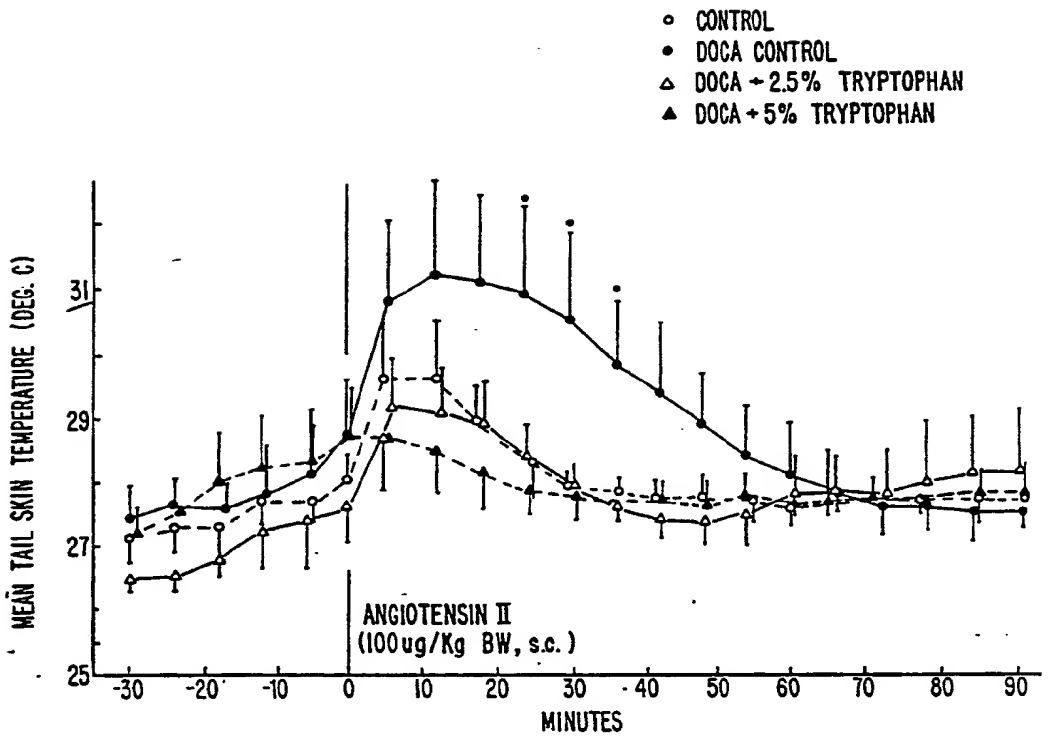

FIG. 7B.

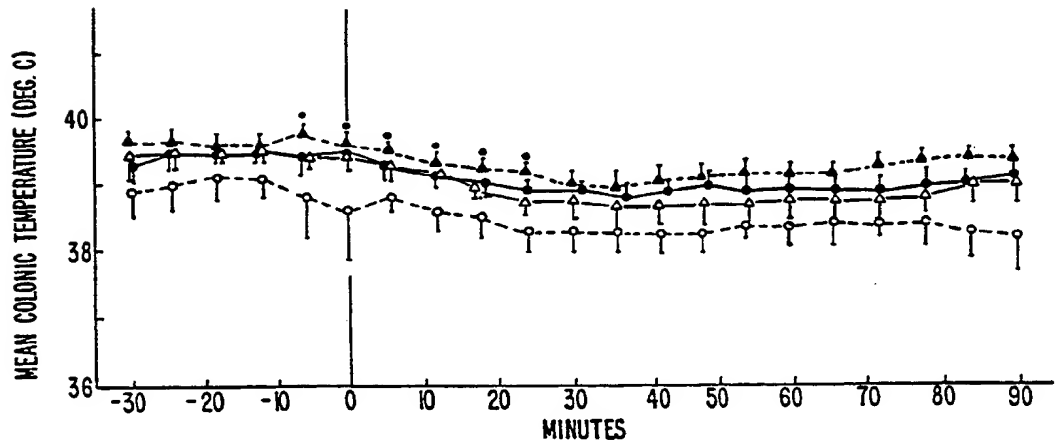

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FIG. 8.

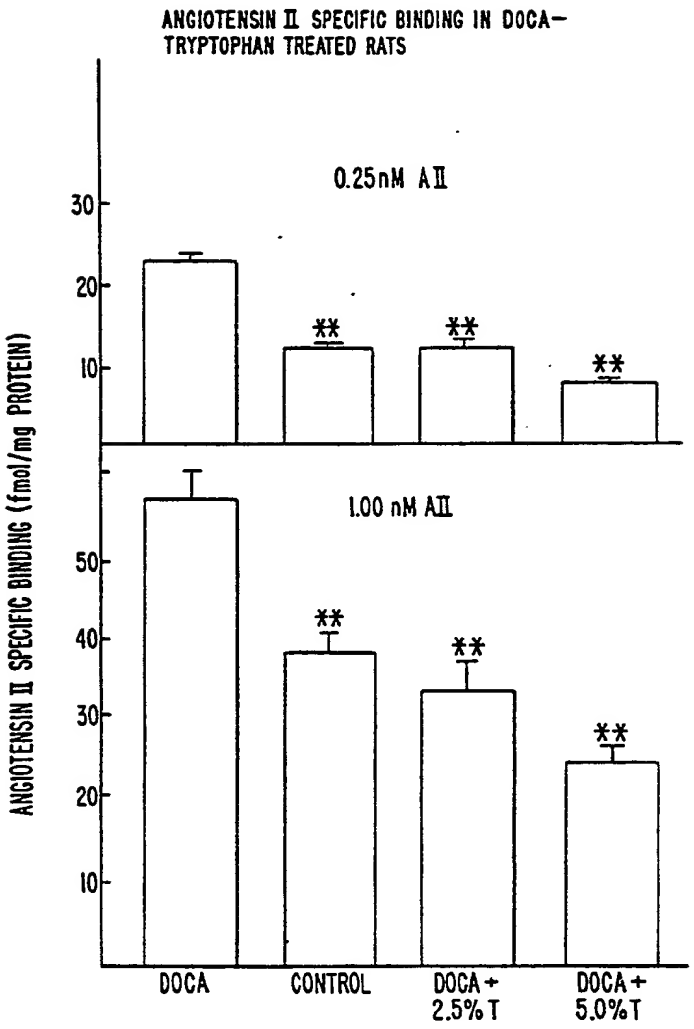

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FIG. 9a.

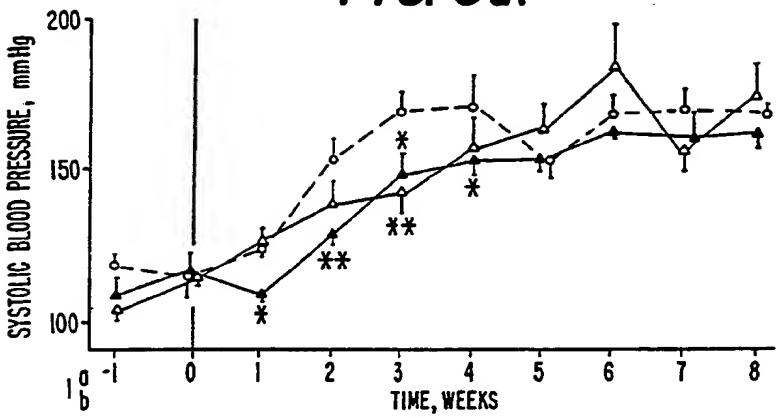

FIG. 9b.

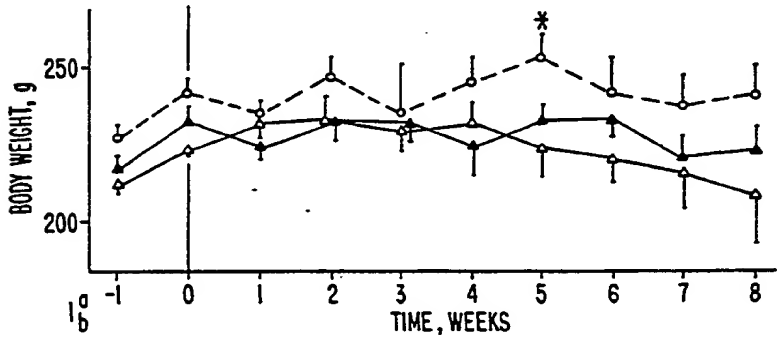

FIG. 10a.

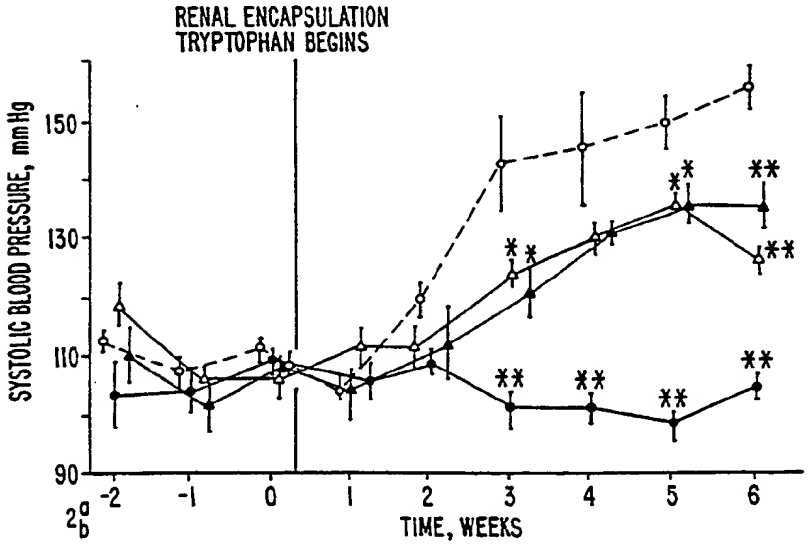

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FIG. 10b.

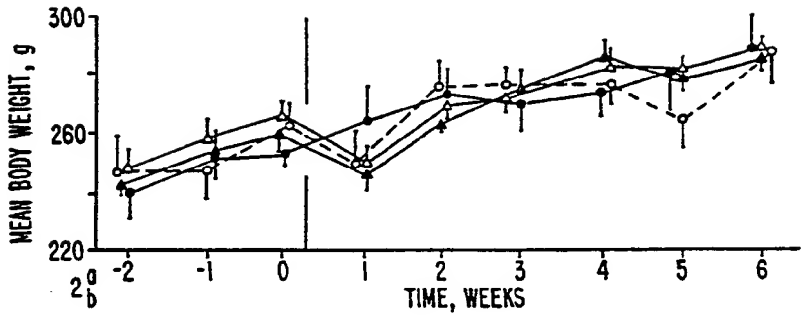
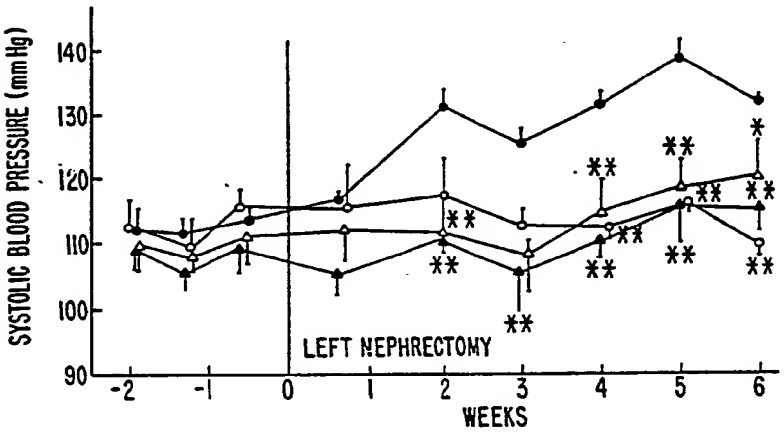


FIG. 11a.



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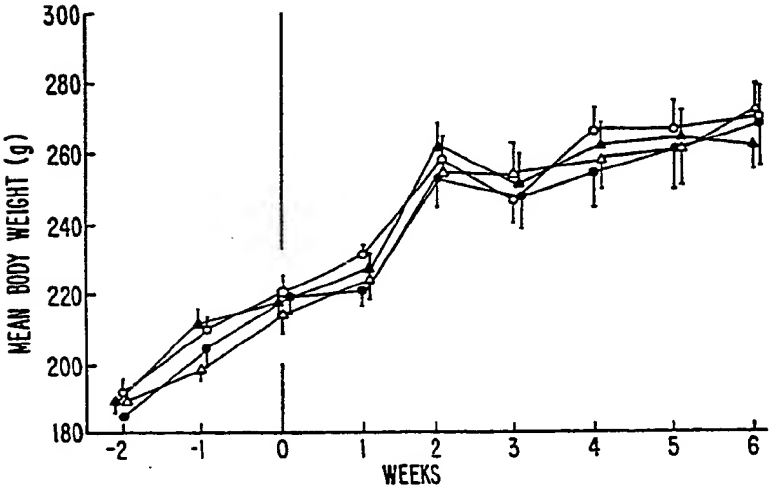

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FIG. 12.

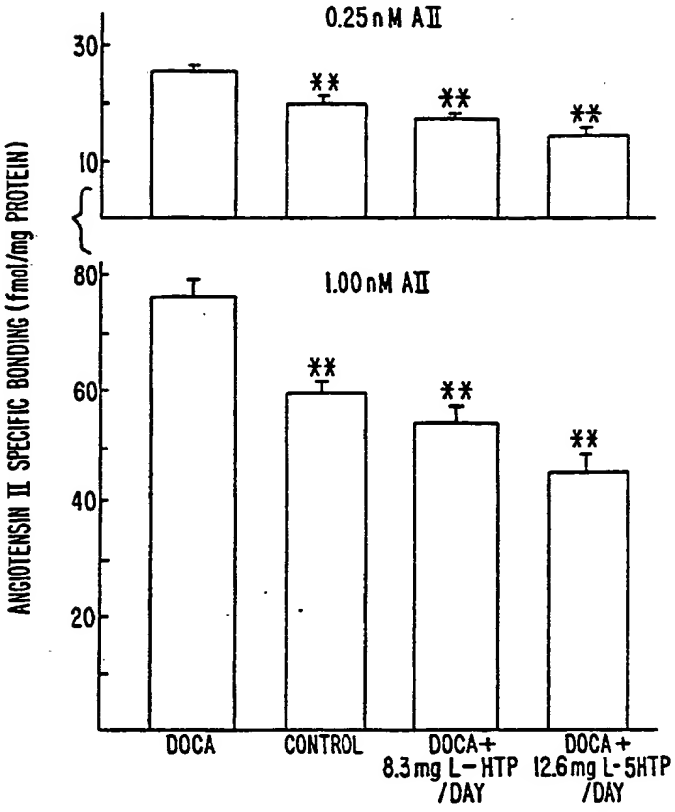

FIG. 13a.

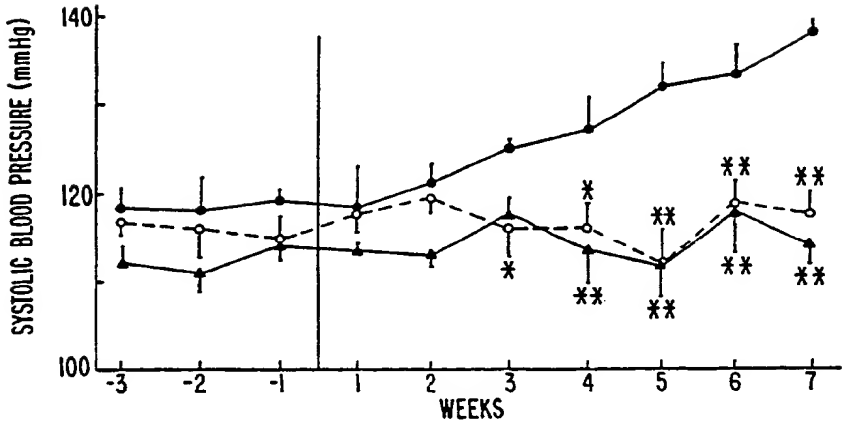

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FIG. 13b.

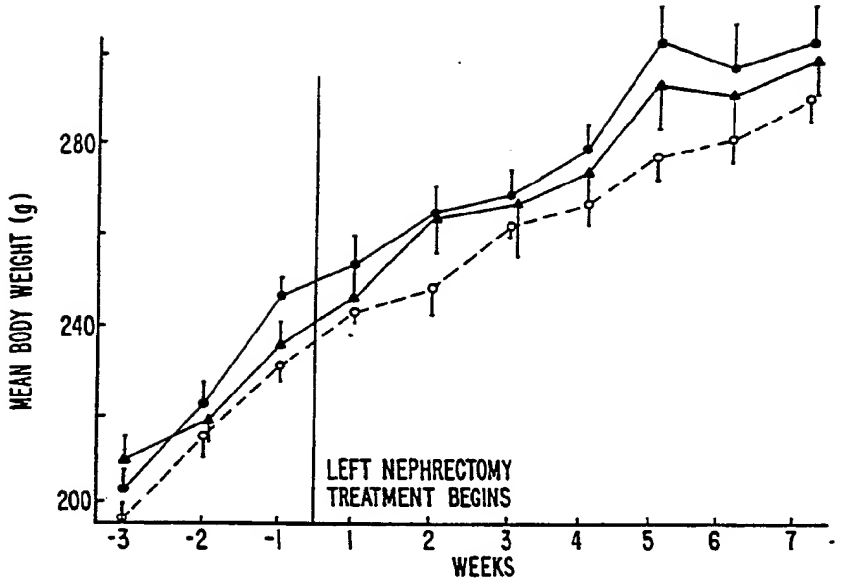

FIG. 14a. FIG. 14b.

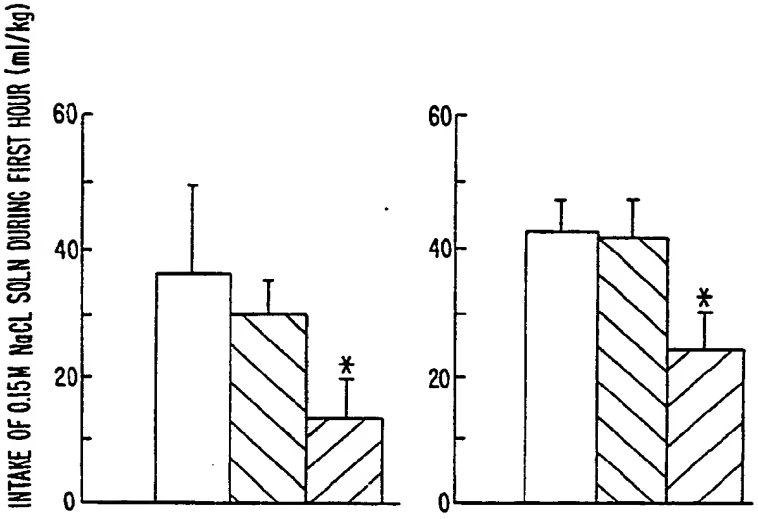

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FIG. 15.

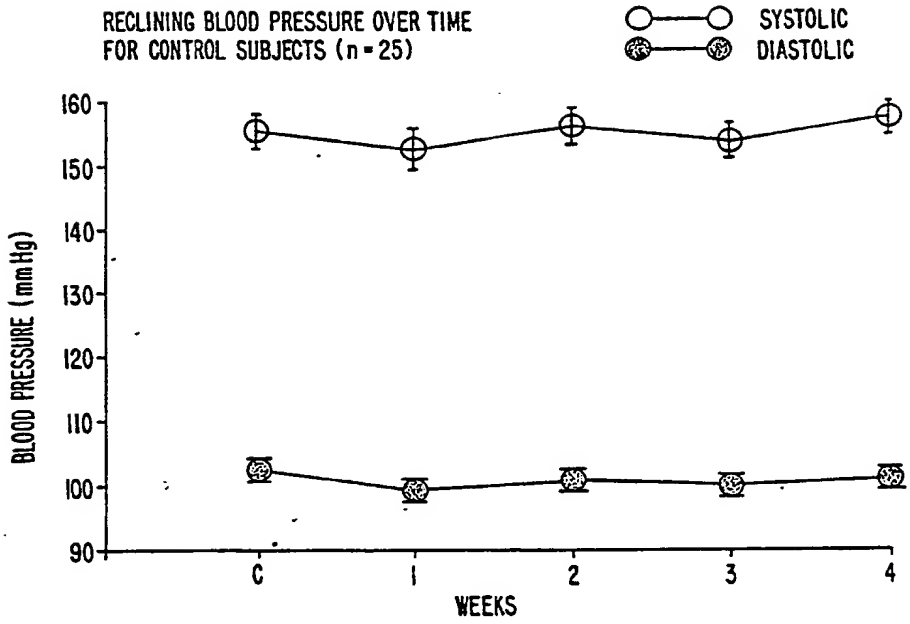

FIG. 16.

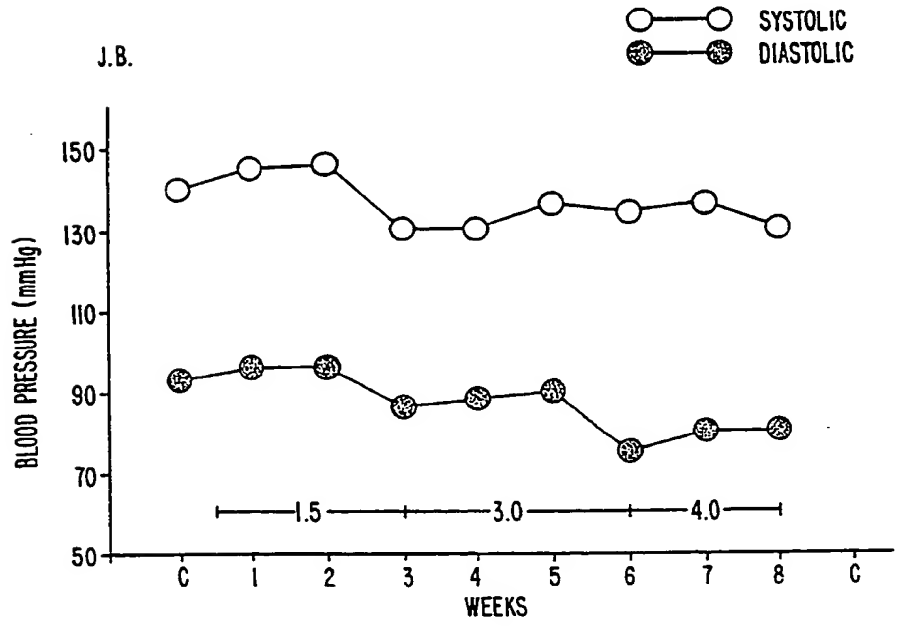

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FIG. 17.

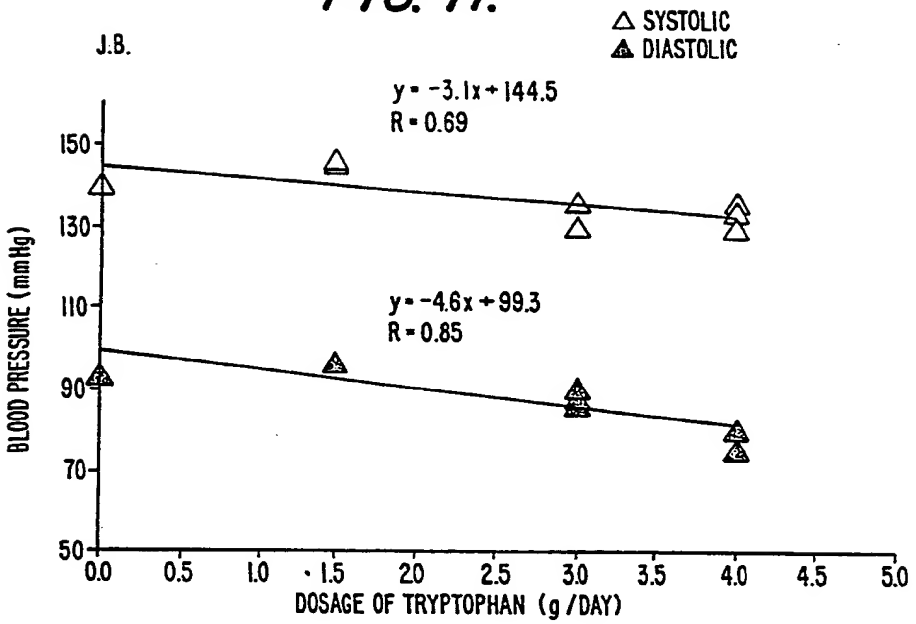

FIG. 18.

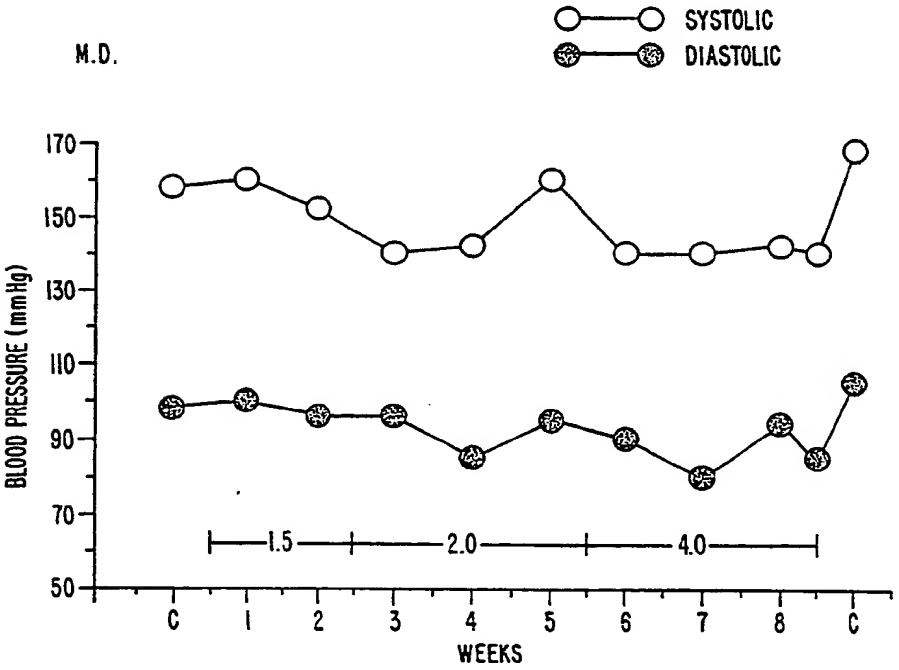

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FIG. 19.

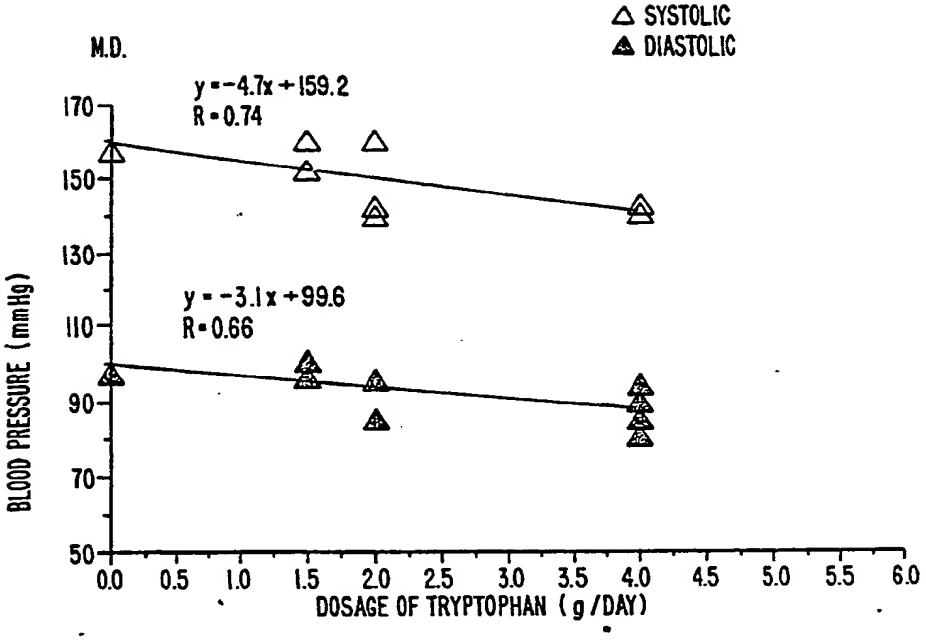

FIG. 20.

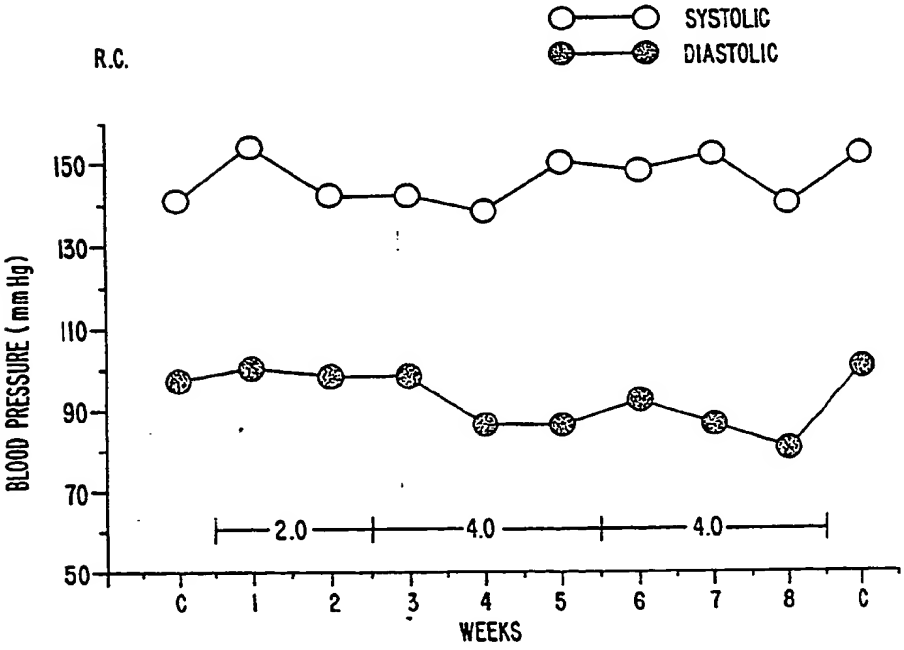

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FIG. 21.

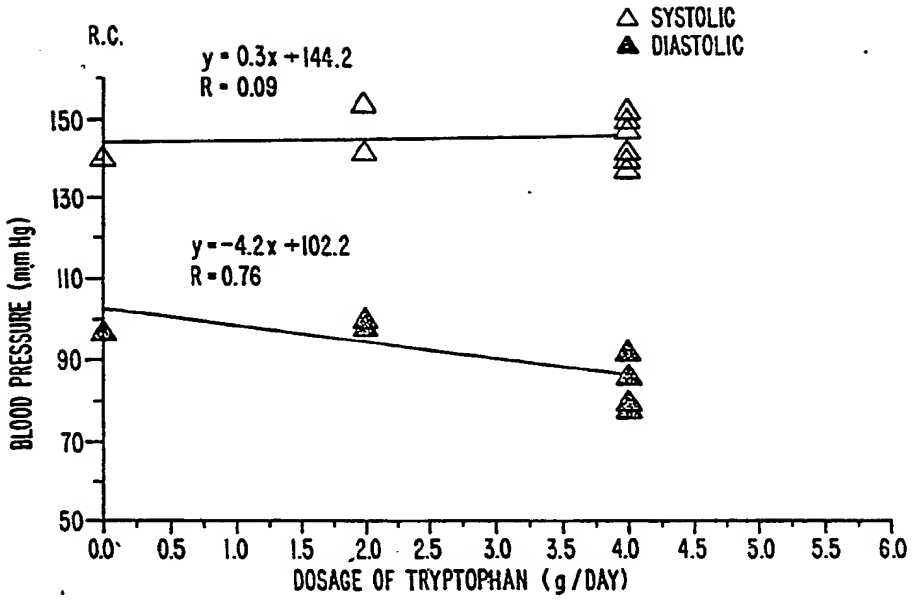
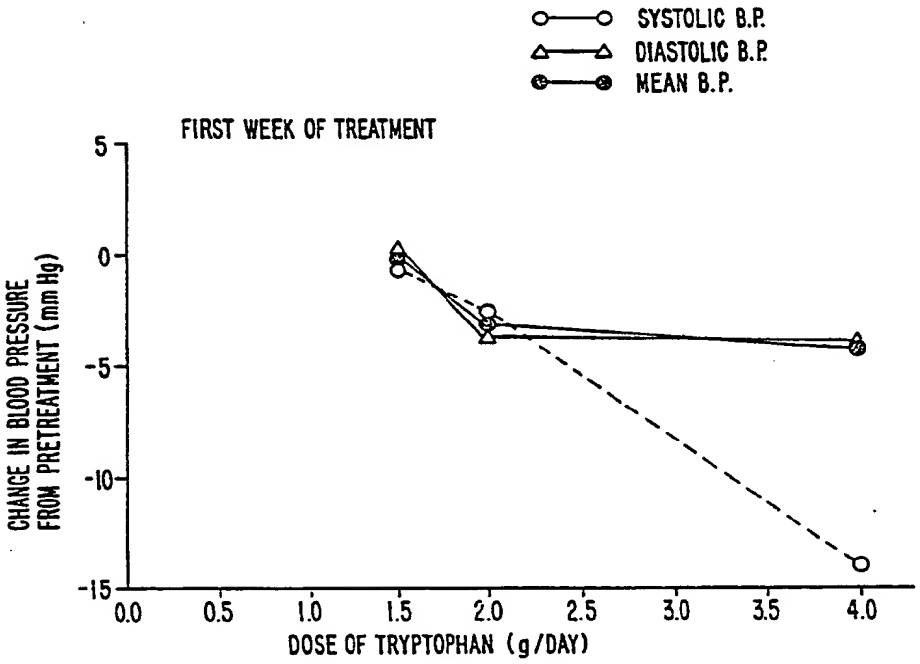


FIG. 22.



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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03302

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC4: A 61 K 31/405; A 23 L 1/30

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC4	A 61 K; A 23 L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 4296119 (RICHARD J. WURTMAN) 20 October 1981, see especially Example III --	15-16
X	US, A, 4329356 (DONALD R. HOLLAND) 11 May 1982, see especially column 1, lines 29-41 --	15-16
X	WO, A1, 85/03873 (ERK VERNON) 12 September 1985, see especially page 16 --	15-16
X	Canadian Journal of Physiology and Pharmacology, Vol. 65, No. 5, 1987 Melvin J Fregly et al.: "Chronic dietary administration of tryptophan prevents the development of deoxycorticosterone acetate salt induced hypertension in rats. ", see page 753 - page 764, especially page 763, right column, third paragraph --	15-16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th January 1989	10 FEB 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Journal of Hypertension, Vol. 4 (suppl. 6), 1986, Francesco Squadrito et al.: "Effect of 5-Hydroxytryptophan on the Development of Hypertension in Spontaneously Hypertensive Rats. ", see pages S254-S256 --	15-16
X	Chemical Abstracts, volume 106, no. 19, 11 May 1987, (Columbus, Ohio, US), M. J. Fregly et al. : "Prevention of DOCA-induced hypertension in rats by chronic treatment with tryptophan. ", see page 43, abstract 149203h, & Clin. Exp. Pharmacol. Physiol. 1986, 13, 767- 76 --	15-16
P,X	Journal of Hypertension, Vol. 5, 1987 (London) Melvin J. Fregly et al.: "Chronic Treatment with L-5-Hydroxytryptophan Prevents the Development of DOCA-Salt-Induced Hypertension in Rats. ", see pages 621-628, especially pages 626-627 (Discussion) --	15-16

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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-14, because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by therapy /PCT Rule 39 (iv)/.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 88/03302

SA 24869

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 02/11/88
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4296119	20/10/81	EP-A- 0005057	31/10/79
		EP-A-B- 0005058	31/10/79
		EP-A-B- 0005333	14/11/79
		JP-A- 54145220	13/11/79
		JP-A- 54145219	13/11/79
		JP-A- 55007261	19/01/80
		CA-A- 1119956	16/03/82
		US-A- 4327112	27/04/82
		CA-A- 1140858	08/02/83
		CA-A- 1140859	08/02/83
US-A- 4329356	11/05/82	NONE	
WO-A1- 85/03873	12/09/85	AU-D- 39994/85	24/09/85
		EP-A- 0181342	21/05/86
		JP-T- 61501565	31/07/86

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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